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RNA-Seq

Lecture Outline

- **Acknowledgements**
- **Computational Biology Initiative**
- Course goals/outline
- Cluster test
- Sequencing technologies
- Planning an RNA-seq experiment
- Library prep
- Design exercises
- Some helpful resources

Acknowledgements & Sources

Wash U Genome Center
Vince Magrini, Jason Walker, Sean McGrath
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C. Titus Brown
University of Virginia
Aaron Quinlan
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Lax Iyer, Peter Castaldi, CBI

CBI: What do we do?

- The Computational Biology Initiative (CBI) is a forum for Tufts researchers to collaborate and develop competitive grants in ‘omics research.
- Beta Site: sites.tufts.edu/cbi
- Please send us your comments about what you would like to see here!

CBI Computational Biology Initiative

Our Partners

Tufts Medical Center

Tufts University Core Facility

Tufts Center for Neuroscience Research

Tufts CTSI Tufts Clinical and Translational Science Institute

University Information Technology

Provost's office: Tufts Collaborates! and Tufts Innovates! Grant Awards
Kirby Johnson @Vice Provost Office

CBI Computational Biology Initiative

Specific Aims

- **Establish**
  - **A core group of CBI experts**
  - **Computational Biology resources**
    - **dynamic web presence and computing resources**
- **Raise awareness and educate researchers in genomics**

CBI Computational Biology Initiative

How can we work together?

- Discuss your research projects
- Ideas for Symposium/talks/courses
- Contribute to our website: Your ideas, protocols/how to
- Keeping up to date with developments
- **Developing new scientific collaborations across disciplines!**

CBI Computational Biology Initiative

More about CBI

- Attend an open meeting of CBI to discuss how we could work together and what needs to be done!
- Easiest way to contact: lax.iyer@tufts.edu

  - Peter Castaldi, TMC
  - Larry Parnell, HNRCA
  - Gordon Huggins, TMC
  - Gavin Schnitzler, TMC
  - Joshua Ainsley, Tufts
  - Lionel Zupan, Tufts

CBI Computational Biology Initiative

Who am I?

Postdoc in Leon Reijmers' lab

Research focused on translation of mRNA in neuronal dendrites

Attended the CSHL Advanced Sequencing Technologies course in 2010

Practical experience in molecular biology and computational methodologies for RNA-Seq

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Course Goals

You will learn how to:

- Prepare an RNA-Seq library
- Assess the quality of your RNA-Seq data
- Utilize basic Unix and R programming
- Align RNA-Seq reads to appropriate reference sequences
- Quantify gene and transcript expression
- Perform differential expression analysis

Course goals (for real)

You will have some idea of where to start.

Course Outline

Day 1: Sequencing technologies, Library preparation, Experiment planning

Day 2: Unix introduction, File formats, Assessing sequence quality

Day 3: Sequence alignment, Read visualization, Expression quantification

Day 4: R introduction, Differential expression analysis

Day 5: Open workshop: Assembly, alternative splicing, RNA editing

Course Methodology

Lectures to introduce concepts

Hands on exercises – Participate!

Teamwork! Yes!

Minute cards

- What did you learn?
- What are you confused about?

Course website:
http://sites.tufts.edu/cbi/resources/rna-seq-course/

Why should you learn Unix/R?

Galaxy is a wonderful tool,
but it can't do everything...

Text manipulation

Adopt new methods

Biology is becoming a data-driven science

Bioinformatics is hard

Get used to being frustrated

Hundreds of poorly-maintained programs

Dozens of file formats

Inefficient tab delimited files

Papers without code

Programming is awesome

Access to newest technology and methods

Automation of tasks

Saves time, money, unnecessary collaborations

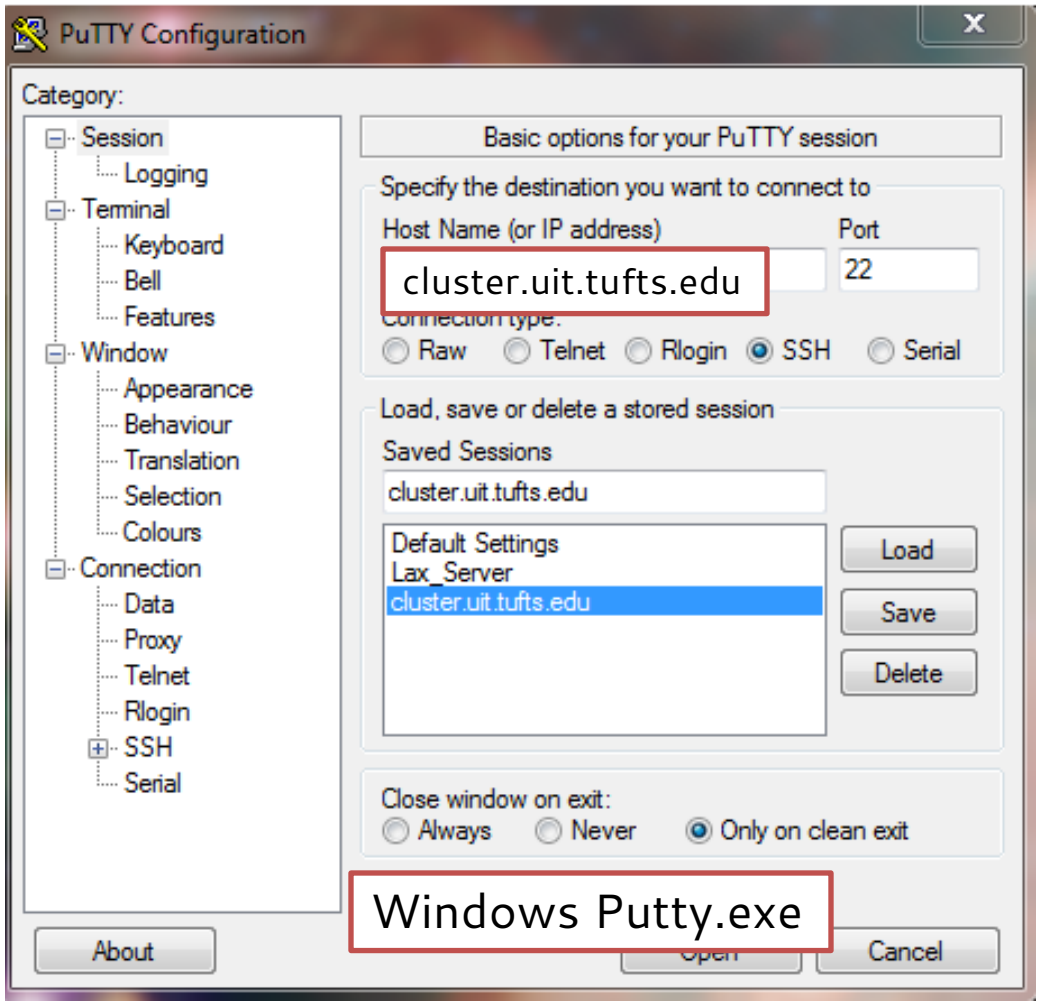
Don't like something? Fix it yourself!
Need something new? Make it yourself!

Amaze your friends and family (and interviewers!)

Bioinformatics job trends

Programming is an effective **tool** you can use to ask the scientific questions you are interested in.

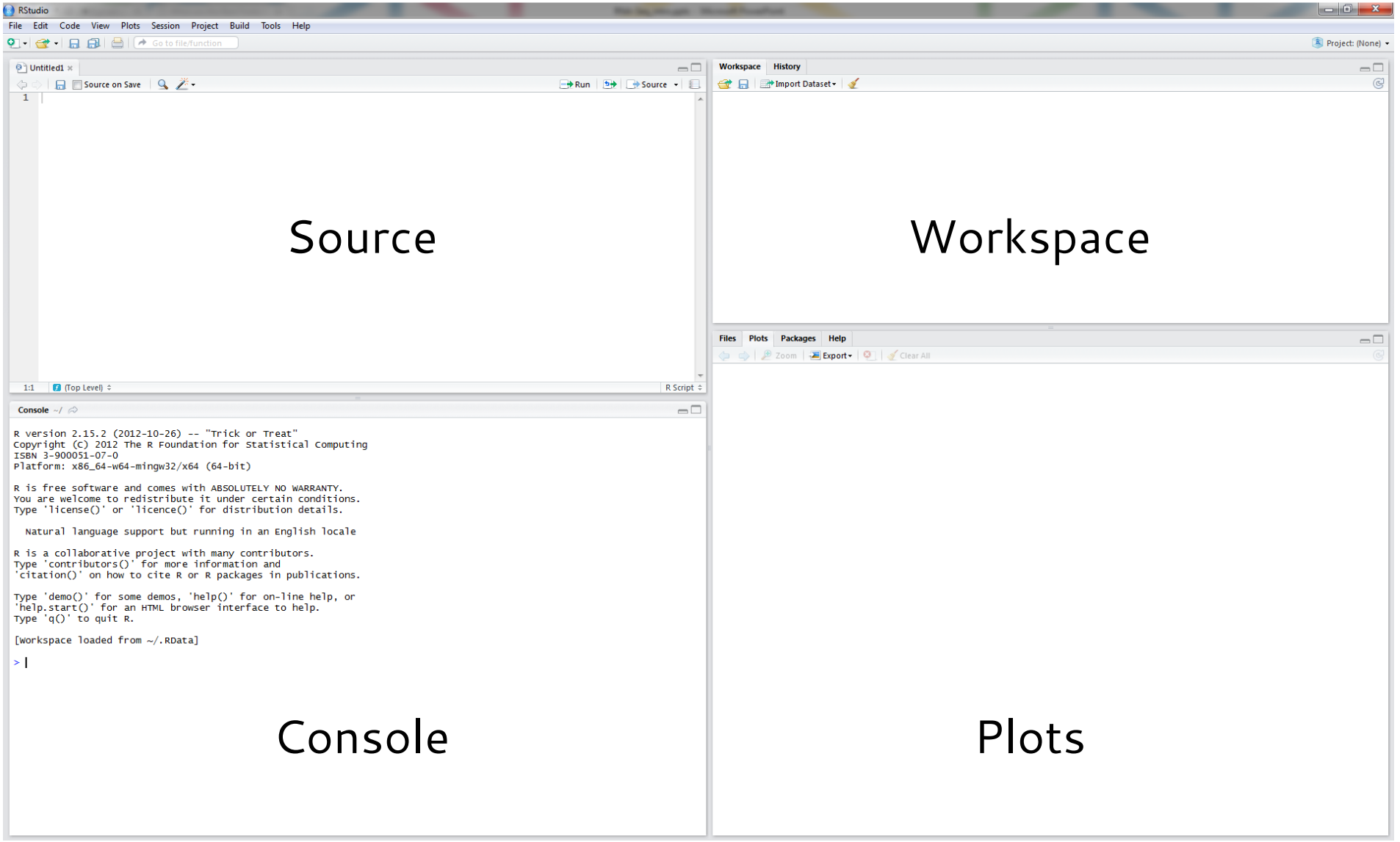
Login to Tufts Cluster



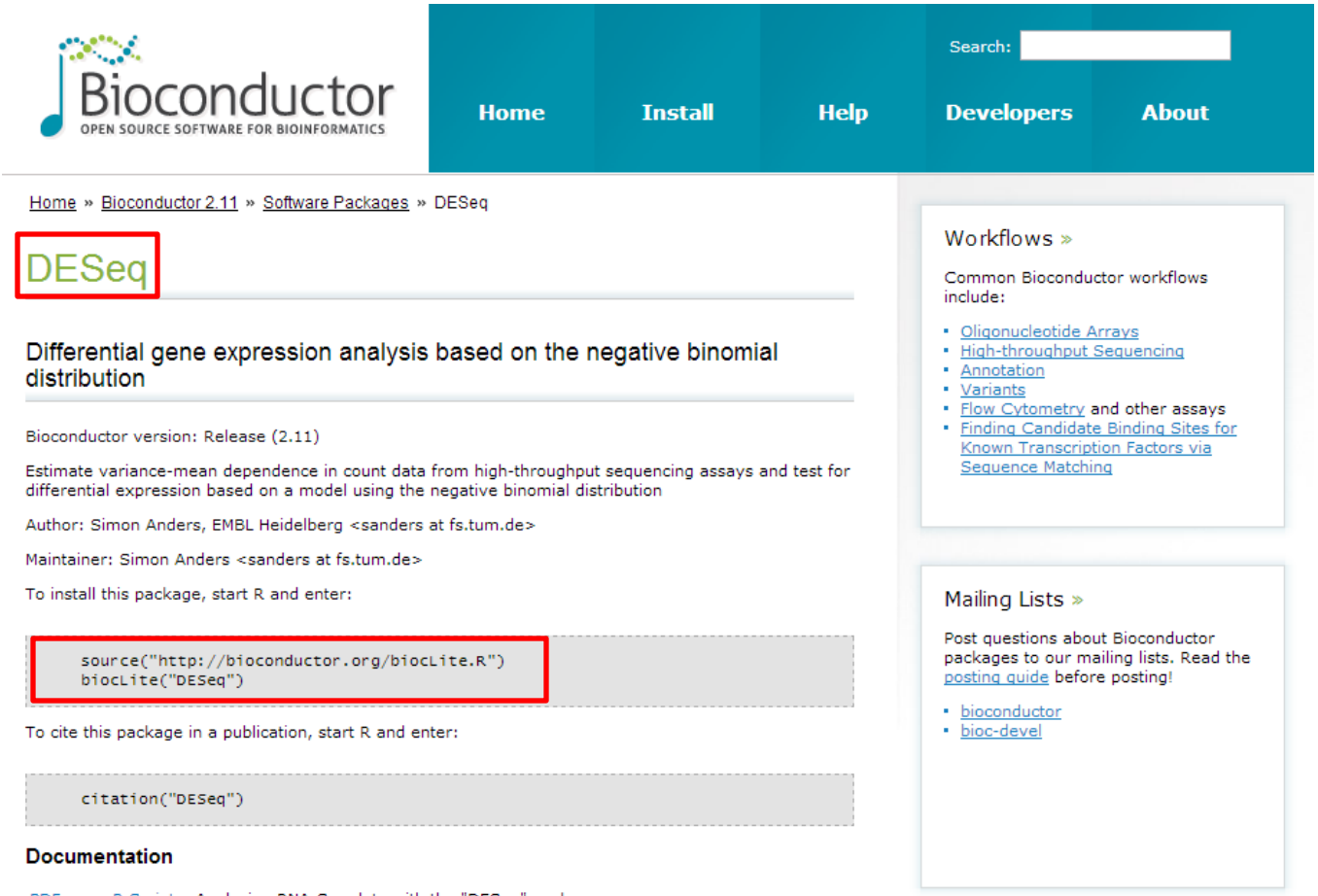
`ssh tuftsid@cluster.uit.tufts.edu`

Integrated Development Environment for R

RStudio



Bioconductor.org



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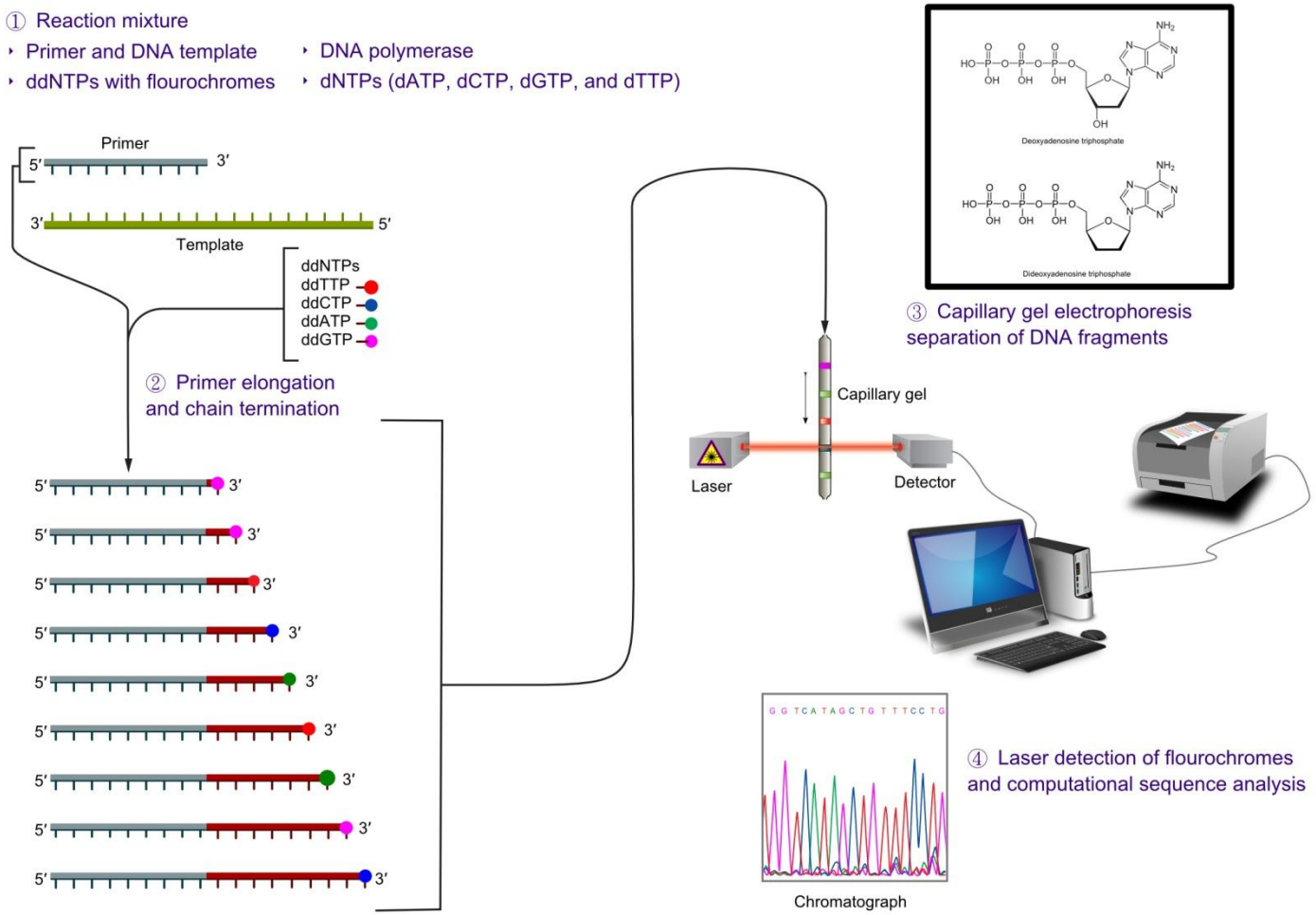
What is next generation sequencing?

First generation = Sanger sequencing



What is next generation sequencing?

Next generation = the stuff that came next

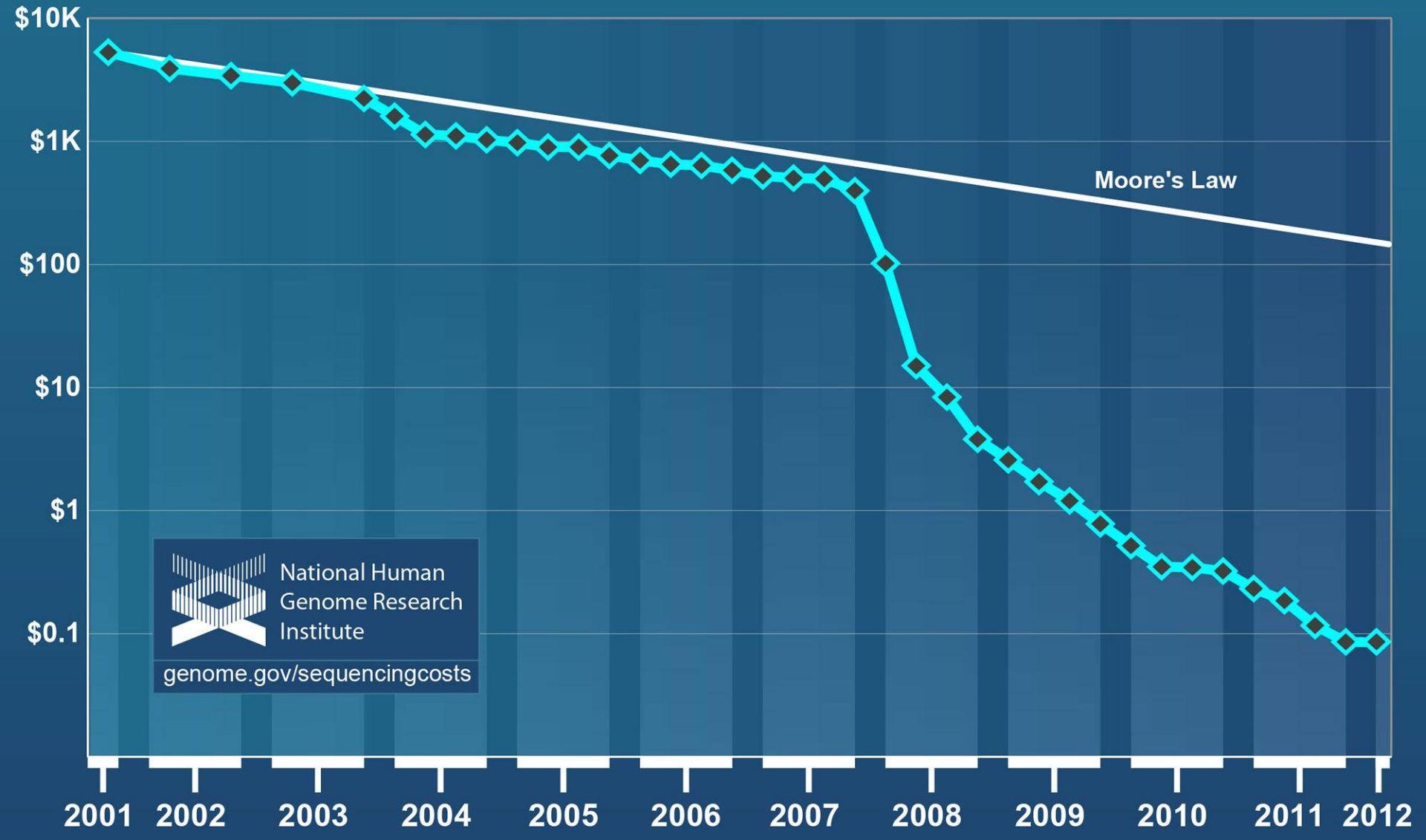
Next generation sequencing(NGS) =
High throughput sequencing(HTS) =
Deep sequencing

It's all just massively parallel sequencing.

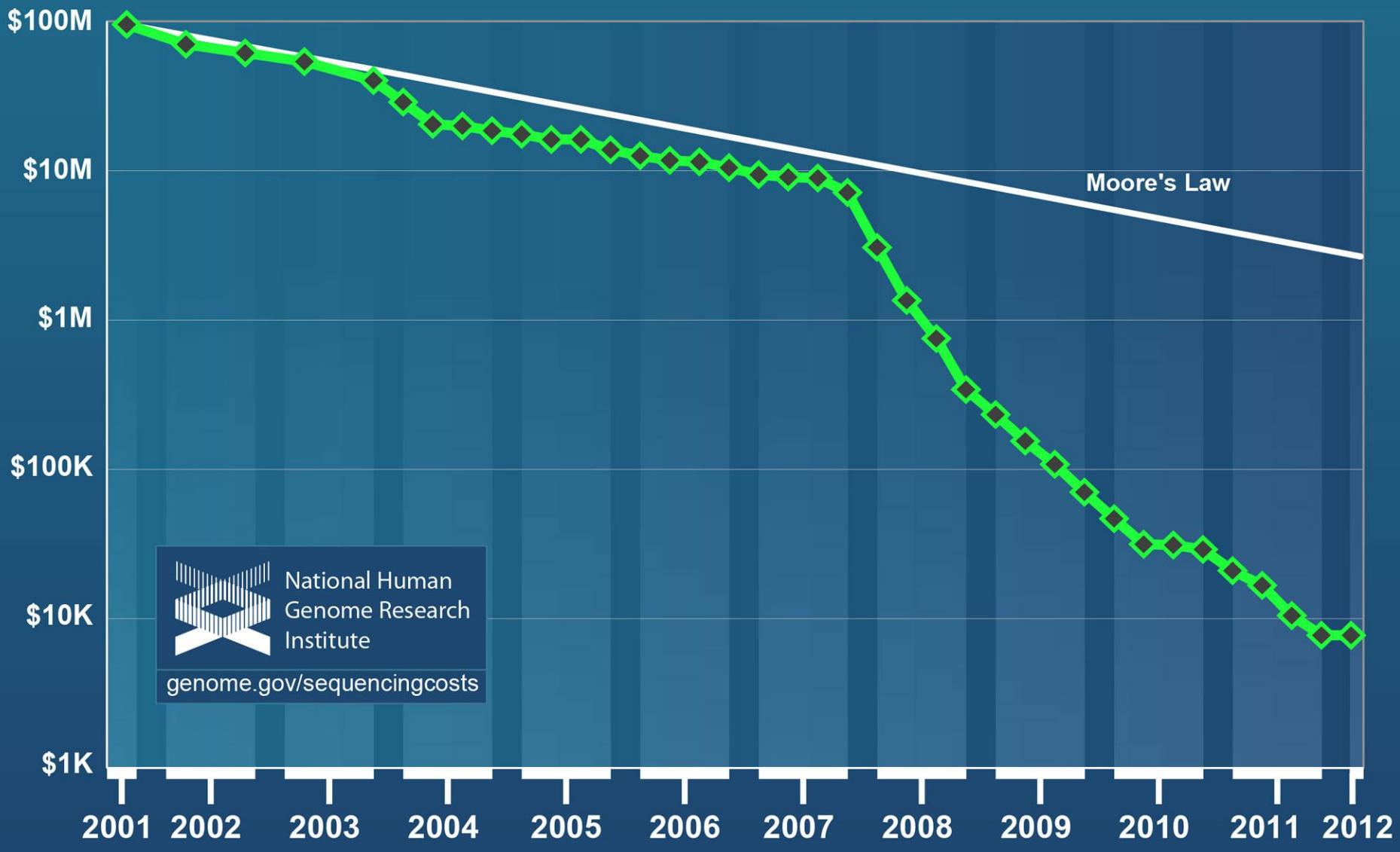
Second generation = Illumina/Solid/454

Third generation = Ion Torrent, Pacific Biosciences, Oxford Nanopore

Cost per Raw Megabase of DNA Sequence



Cost per Genome



HTS Commonalities (so far)

- Fragmentation of starting DNA
- Ligation with custom adapters
- Library amplification on a solid surface (either bead or glass)
- Direct detection of each incorporated nucleotide
- Hundreds of thousands to billions of reactions
- Shorter read lengths than capillary sequencers
- Count based data for quantitation
- Sampling both ends of every fragment sequenced (paired end reads)

Sequencing Platforms

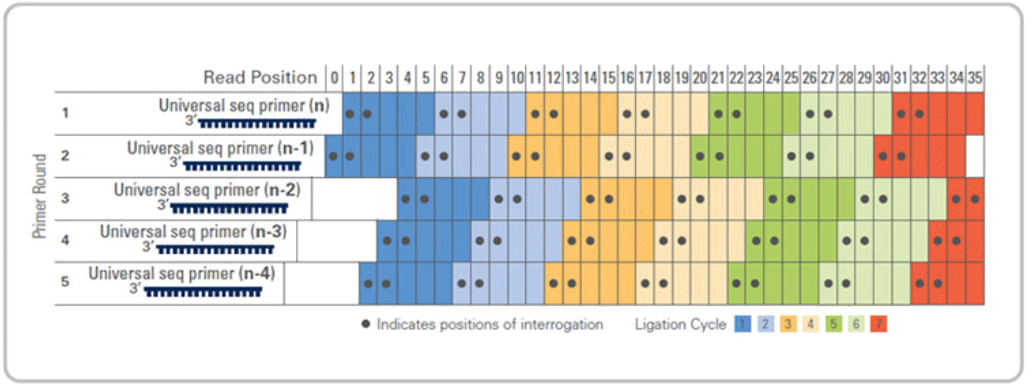
Company	Platform	Amplification	Sequencing
Roche	454	emPCR	Synthesis, Pyrosequencing
Illumina	Illumina/ Solexa	Bridge PCR	Synthesis, Fluorescence
Life	SOLiD	emPCR	Ligation, Fluorescence
Life	Ion Torrent	emPCR	Synthesis, H+ detection
PacBio	RS	None	Synthesis, ZMW fluorescence
Oxford Nanopore	ION	None?	Nanopore current flow

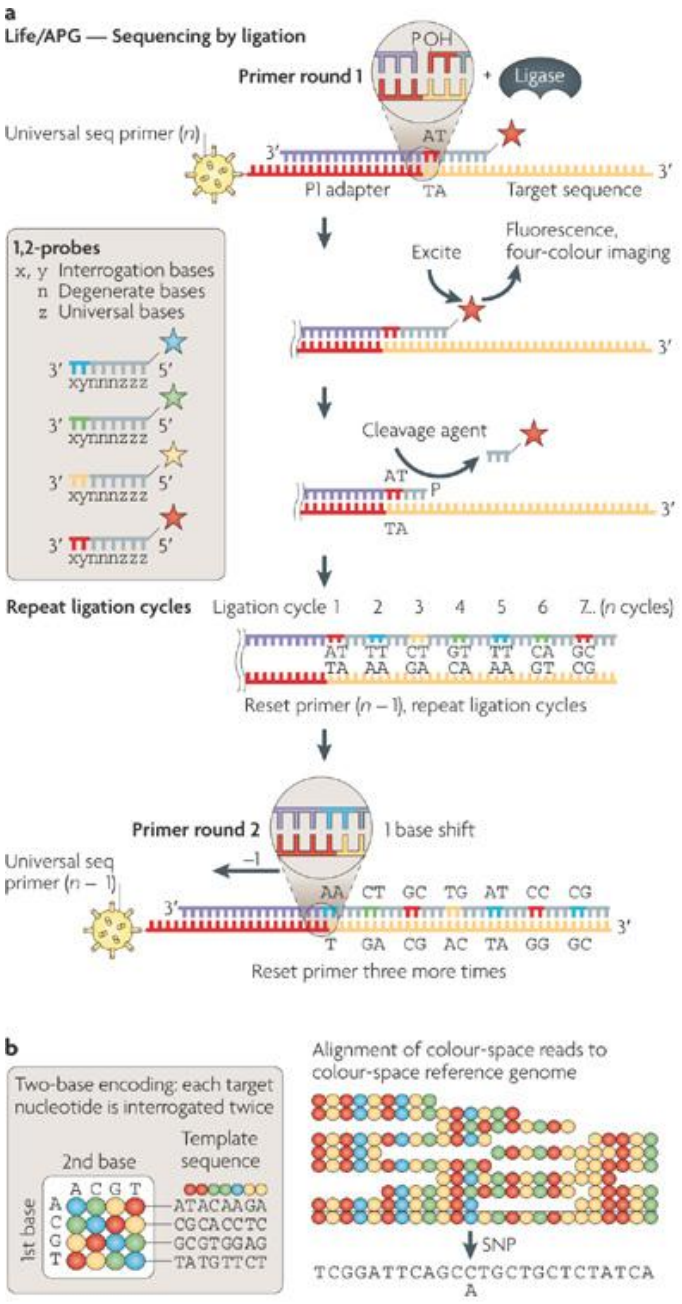
SOLiD Sequencing

Sequencing-by-ligation

Accuracy
(not so much anymore)

Shorter read length



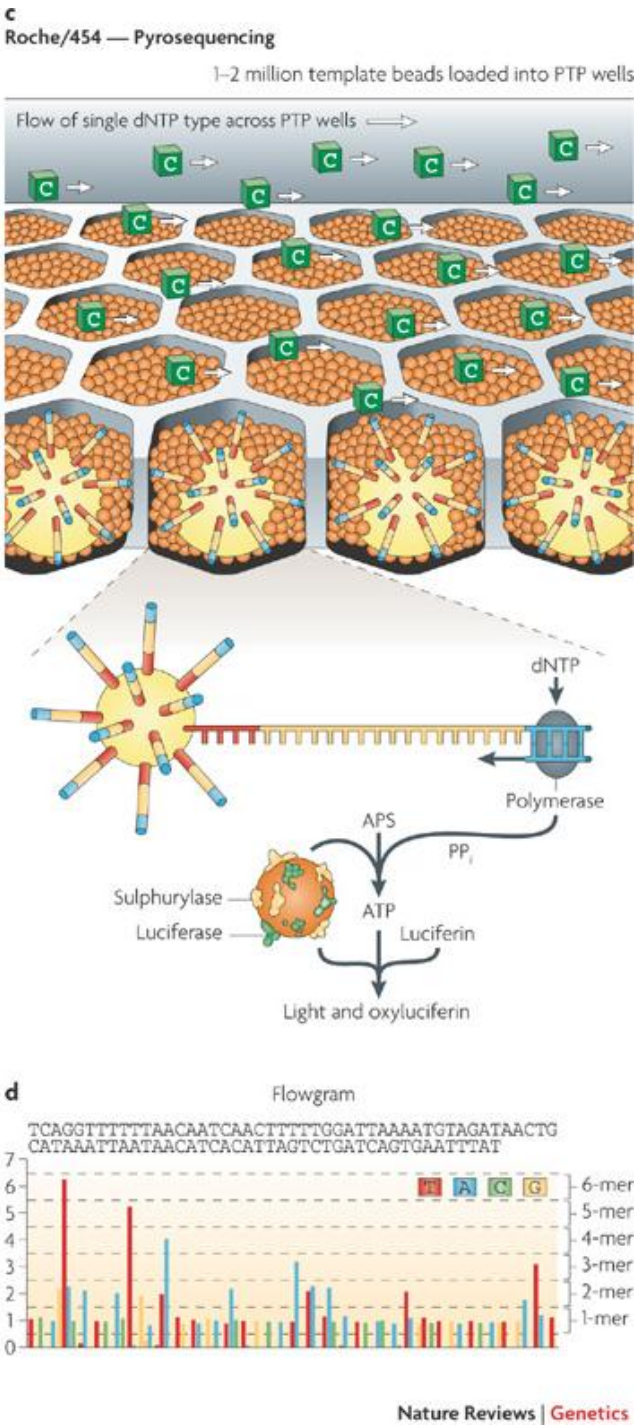


454 Sequencing

Pyrosequencing

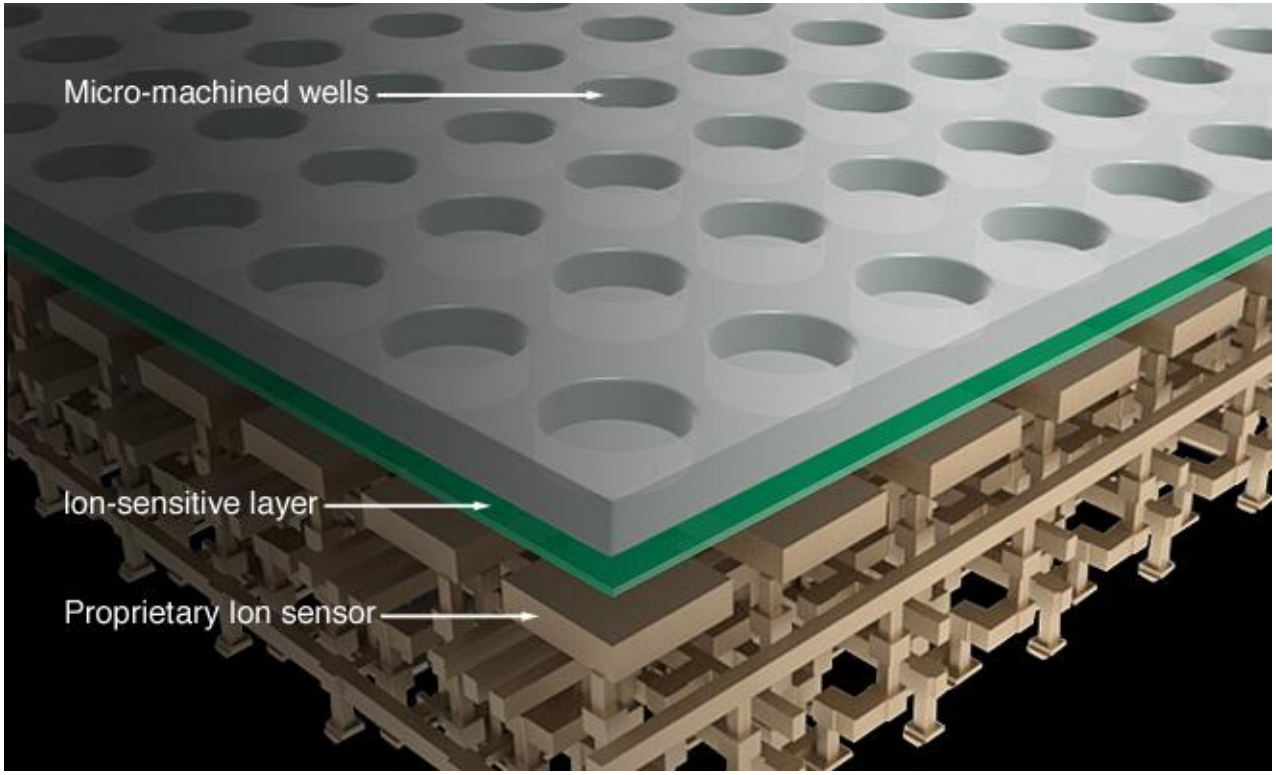
Read Length
(not so much
anymore)

Homopolymer errors



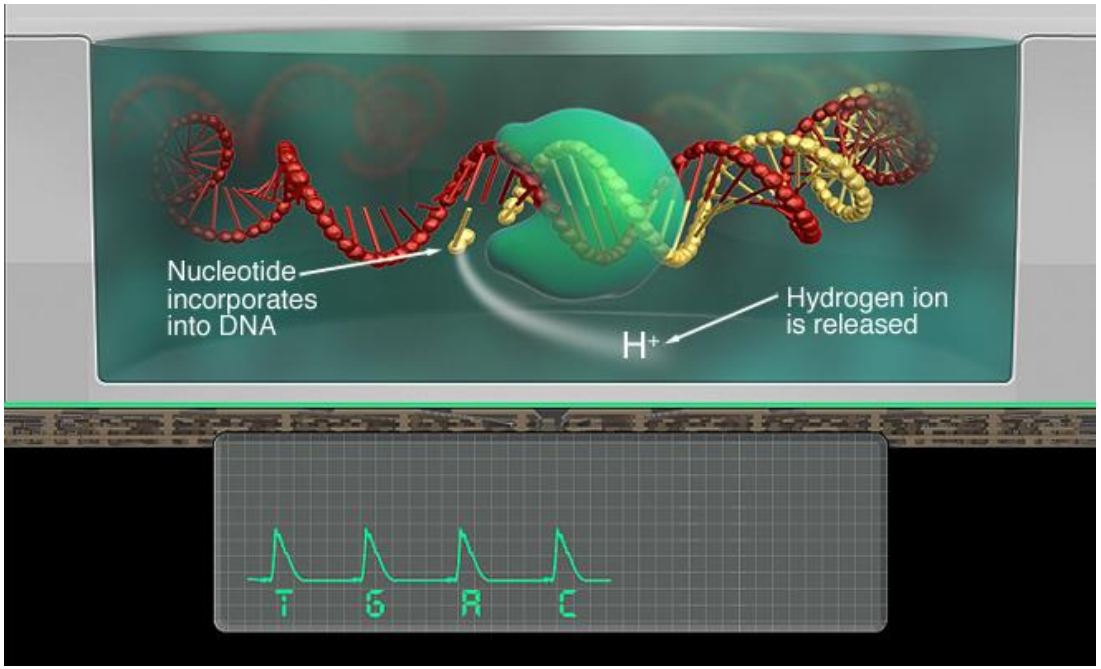
Ion Torrent

Sequencing by detecting the H+ ion released during nucleotide incorporation



Ion Torrent

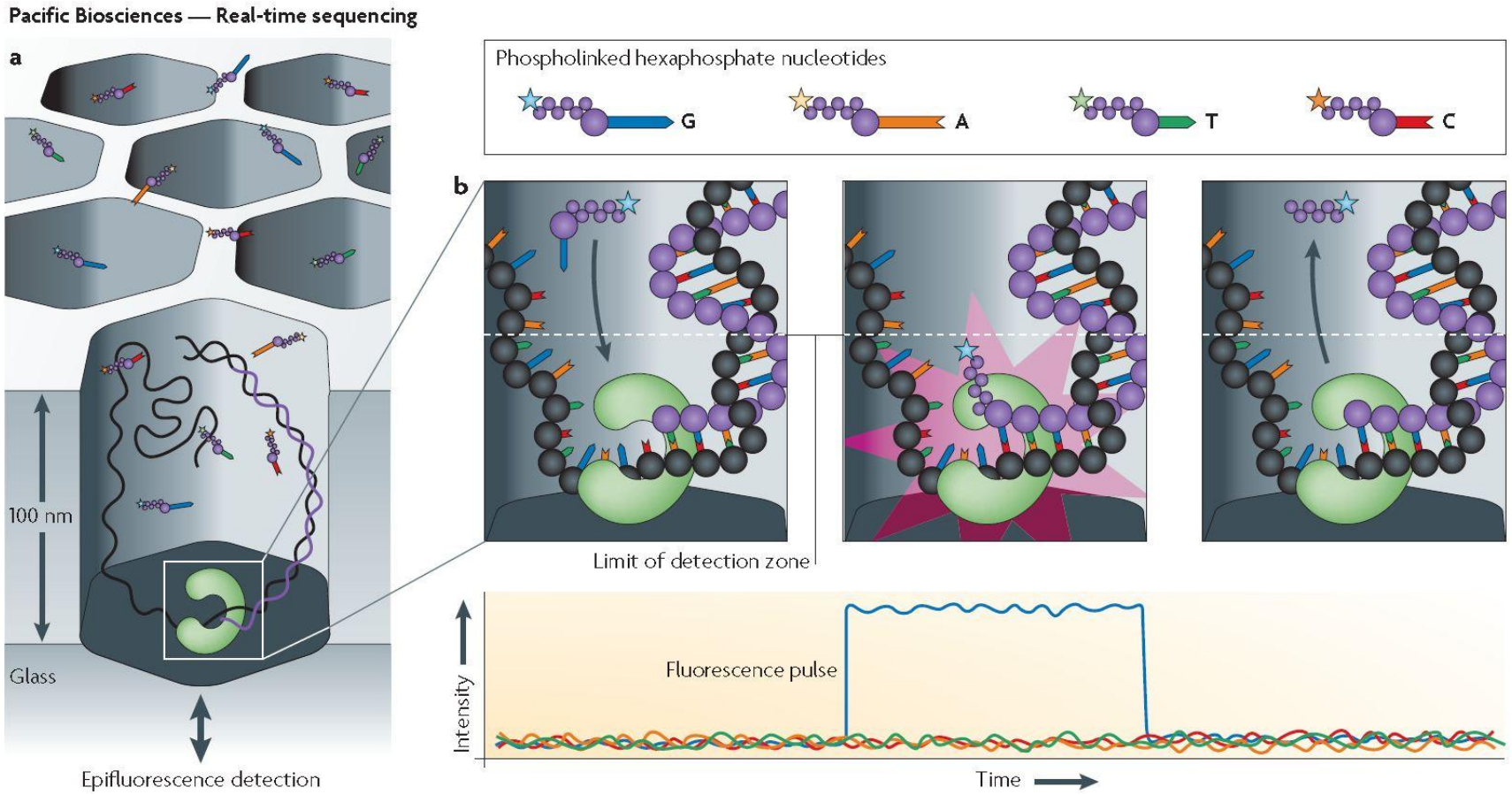
Simple – unmodified nucleotides added one at a time



What error type do you think would be most common in Ion Torrent sequencing?

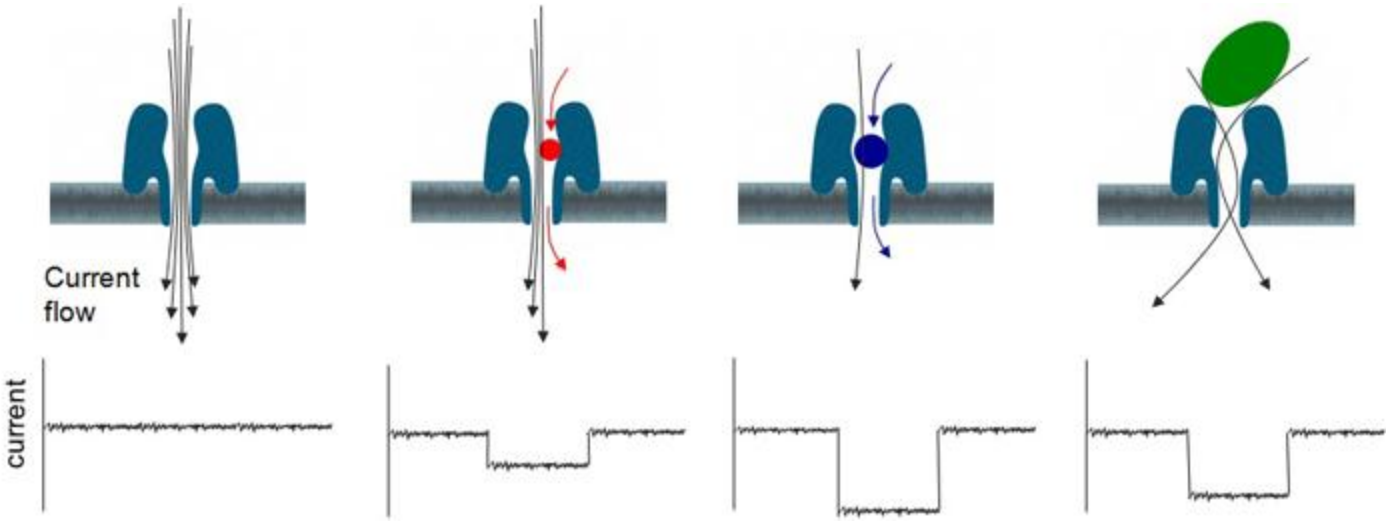
Pacific Biosciences

Real time, zero-mode waveguide sequencing can detect modified bases



Oxford Nanopore

Sequencing by detecting the change in ion flow that occurs when a nucleotide strand travels through a nanopore



Video

GridION and MinION

Illumina Sequencing

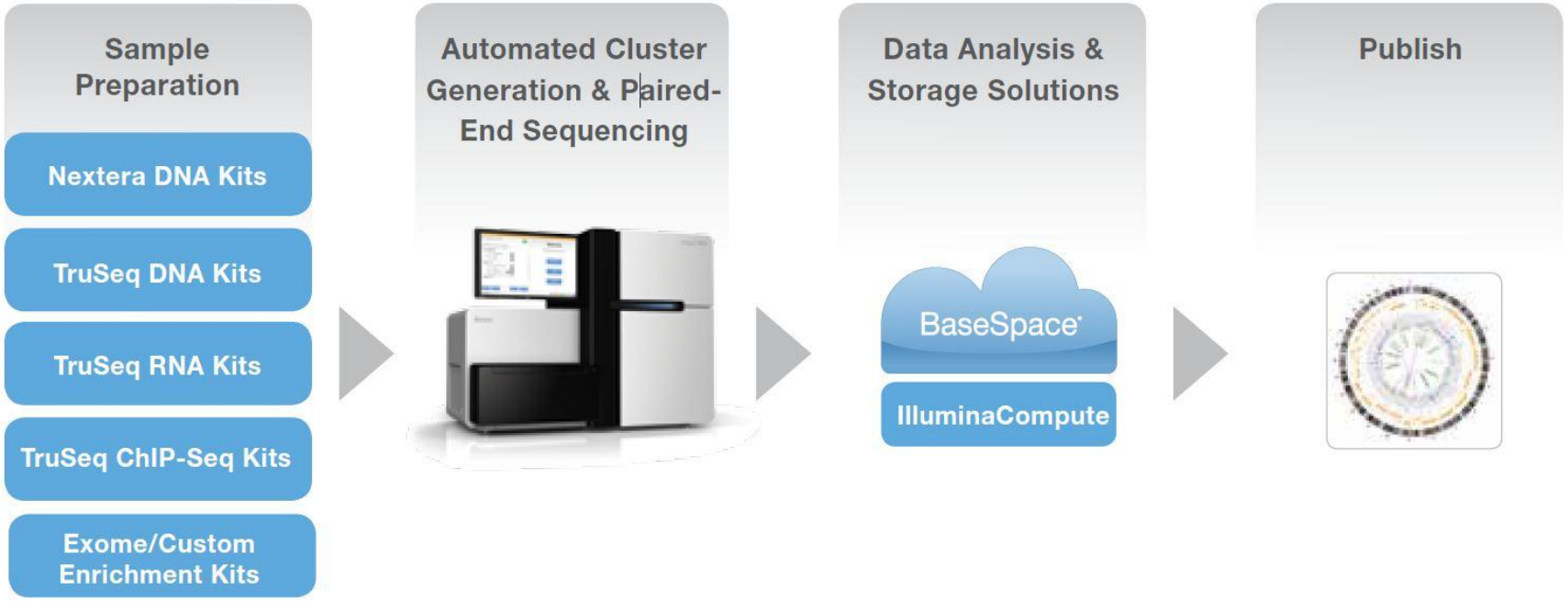
Most widely used sequencing platform

Highest throughput

Increasing accuracy, read length

Large number of third-party
and homebrew reagents

Illumina workflow



Illumina Platforms

HiSeq 2500/1500
Powerful. Flexible. Scalable.

HiSeq 2000/1000
Proven high-throughput platform.

HiScanSQ
Two proven technologies. One powerful platform.

Genome Analyzer IIx
The most widely cited sequencing platform.

MiSeq
The most accurate, easiest-to-use benchtop sequencer in the world.

Output (maximum) 600 Gb	300 Gb	150 Gb	95 Gb	7.8 - 8.5 Gb
Single Reads (maximum) 3 billion total 187 million/lane	1.5 billion total 187 million/lane	750 million total 94 million/lane	320 million total 40 million/lane	15 - 17 million total 15 - 17 million/lane
Paired-end Reads (maximum) 6 billion 374 million/lane	3 billion 374 million/lane	1.5 billion 188 million/lane	640 million 80 million/lane	30 - 34 million total 30 - 34 million/lane
Required input 50 ng with Nextera 100 ng - 1 µg with TruSeq	50 ng with Nextera 100 ng - 1 µg with TruSeq	50 ng with Nextera 100 ng - 1 µg with TruSeq	50 ng with Nextera 100 ng - 1 µg with TruSeq	50 ng with Nextera 100 ng - 1 µg with TruSeq
Read length 2 x 100 bp	2 x 100 bp	2 x 100 bp	2 x 150 bp	2 x 250 bp
Percentage of Bases > Q30 > 85% (2 x 50 bp) > 80% (2 x 100 bp)	> 85% (2 x 50 bp) > 80% (2 x 100 bp)	> 85% (2 x 50 bp) > 80% (2 x 100 bp)	> 85% (2 x 50 bp) > 80% (2 x 100 bp)	> 85% (2 x 100 bp) > 80% (2 x 150 bp) > 70% (2 x 250 bp)

Illumina Platforms

MiSeq

- Clustering on-board
- Fast Chemistry
- Longer Reads

HiSeq 2500

- Genome in a day
- Clustering on-board
- Complete walk-away workflow
- Longer 2x150 reads

HiSeq 2000

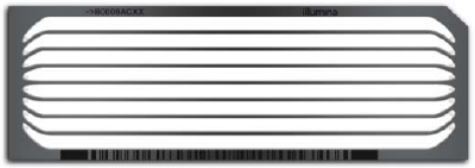
- Data rate
- TDI scanning
- Larger flow cell

Illumina 2500 run modes

1 Instrument – 2 Run Modes

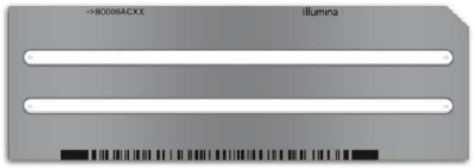
High Output Mode

600 Gb in ~10.5 days
3 billion clusters
cBot required



Rapid Run Mode

120Gb in ~1 day
600 million clusters
No cBot required



User configurable

6 human genomes in 10.5 days

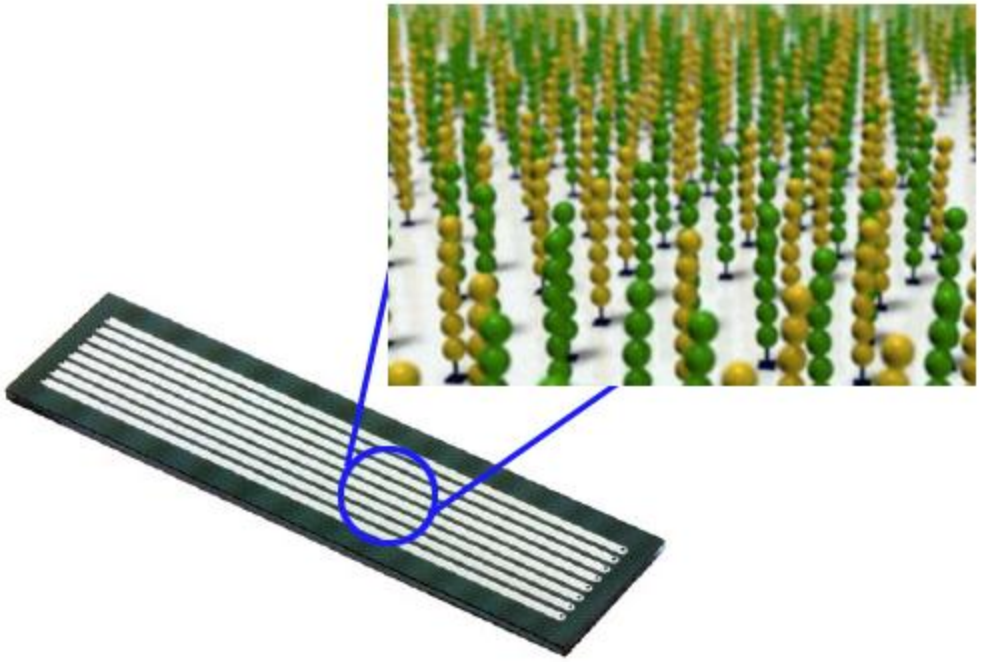
Highest output

1 human genome in a day

Fastest turnaround

Illumina flowcells

microfluidic chambers
for reagent application



Covalently linked oligos

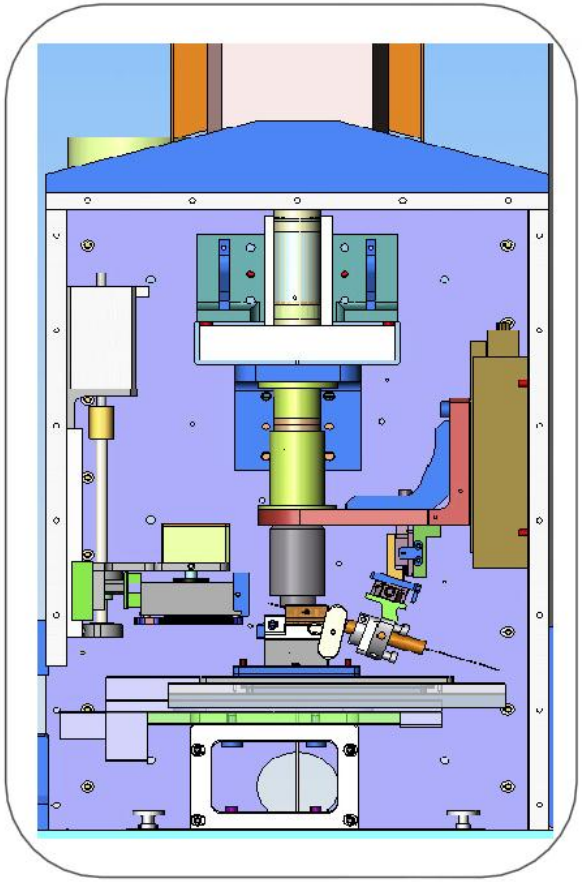
Invariant sample position

Throughput depends on imaging area

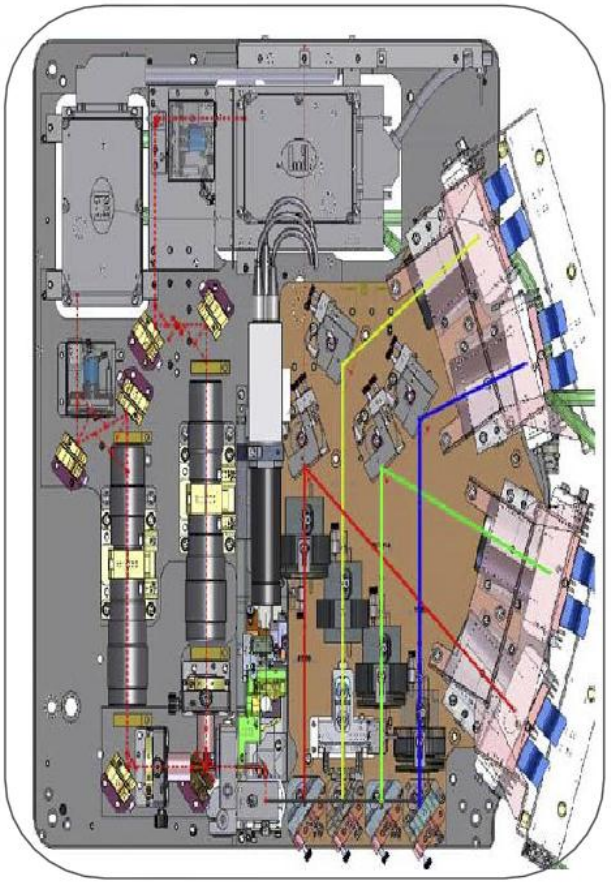
Increases in surface area and imaging area

Illumina Technology

Fluorescent microscopy and microfluidics

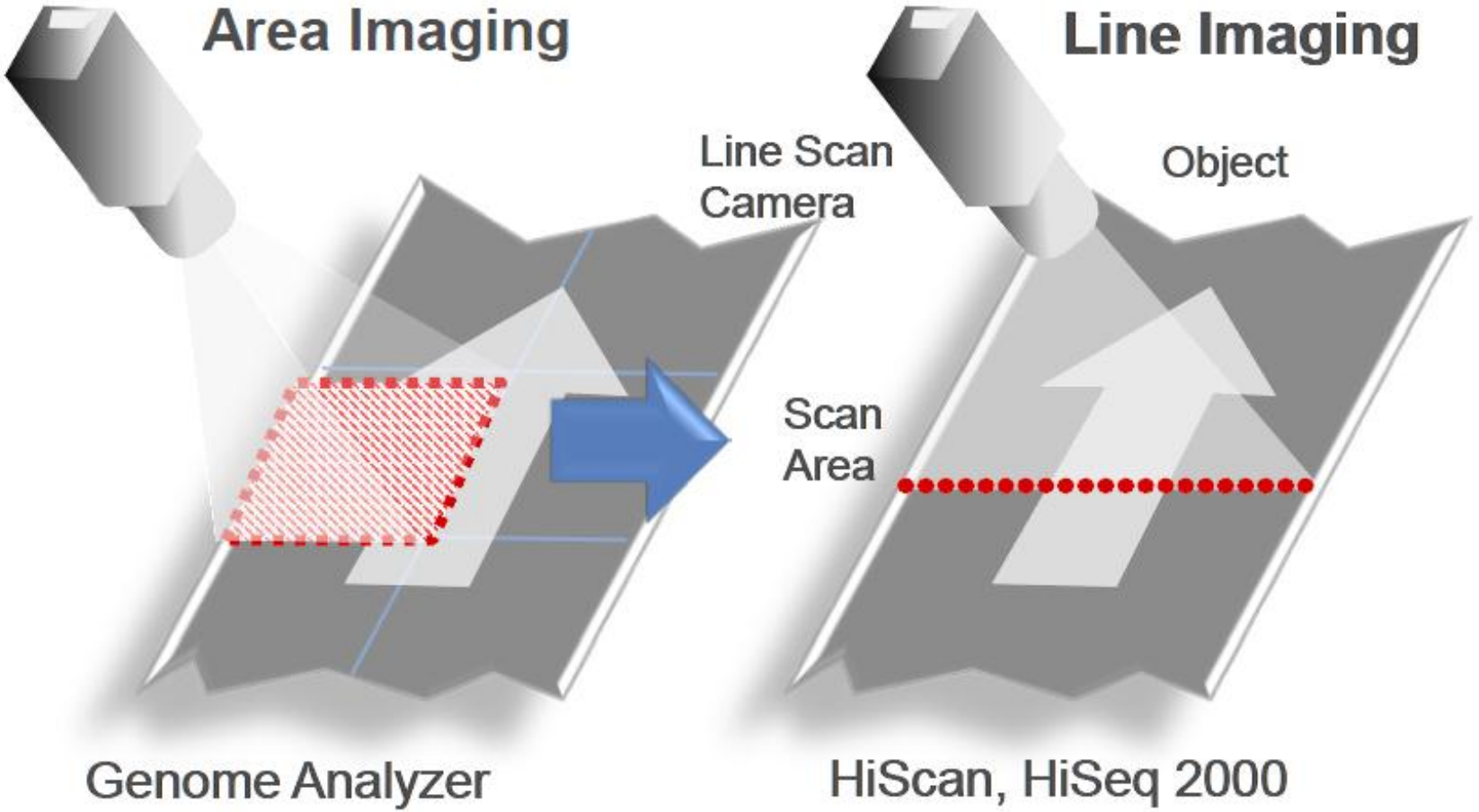


Genome Analyzer



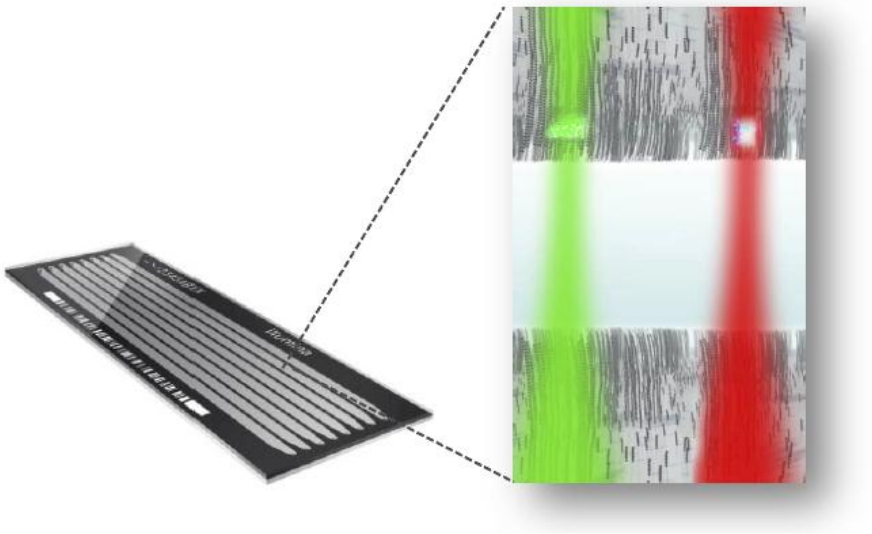
HiSeq 2000

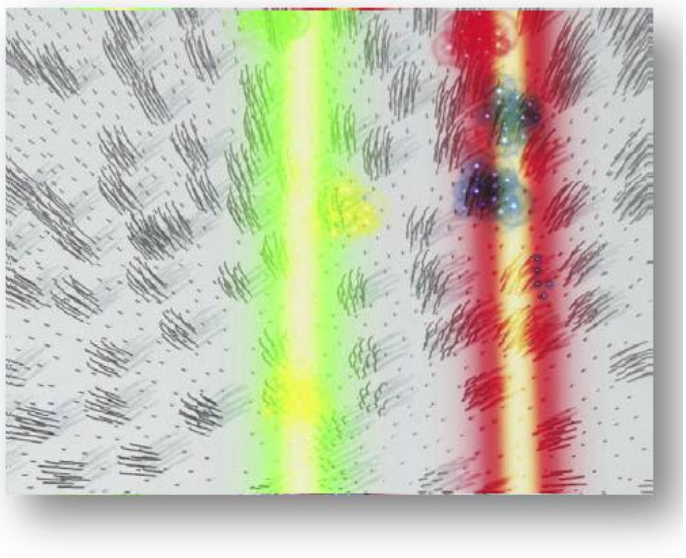
Imaging an Illumina flowcell



Imaging an Illumina flowcell

HiSeq2000 – images bottom and top of flow cell sequentially



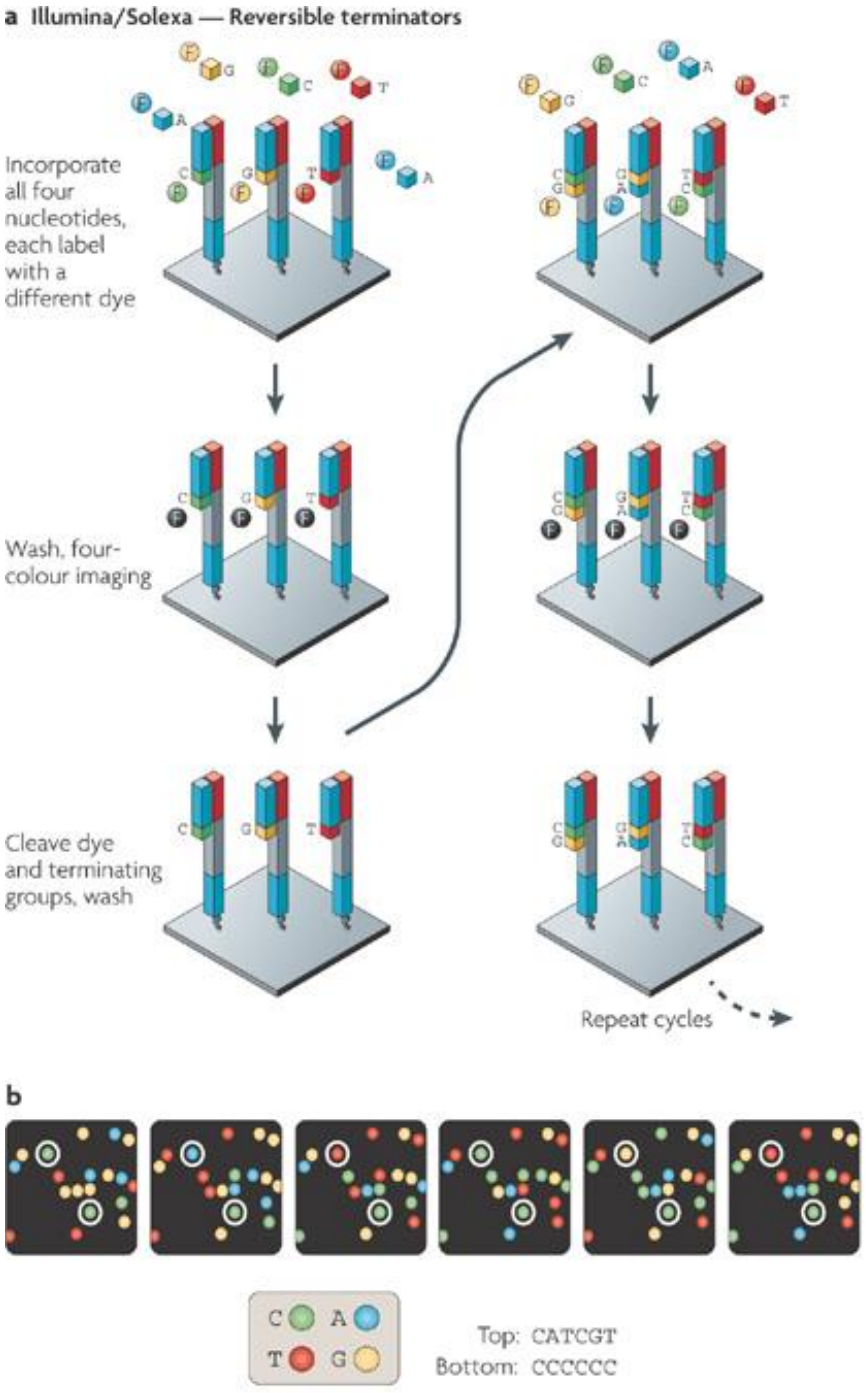


Illumina Sequencing

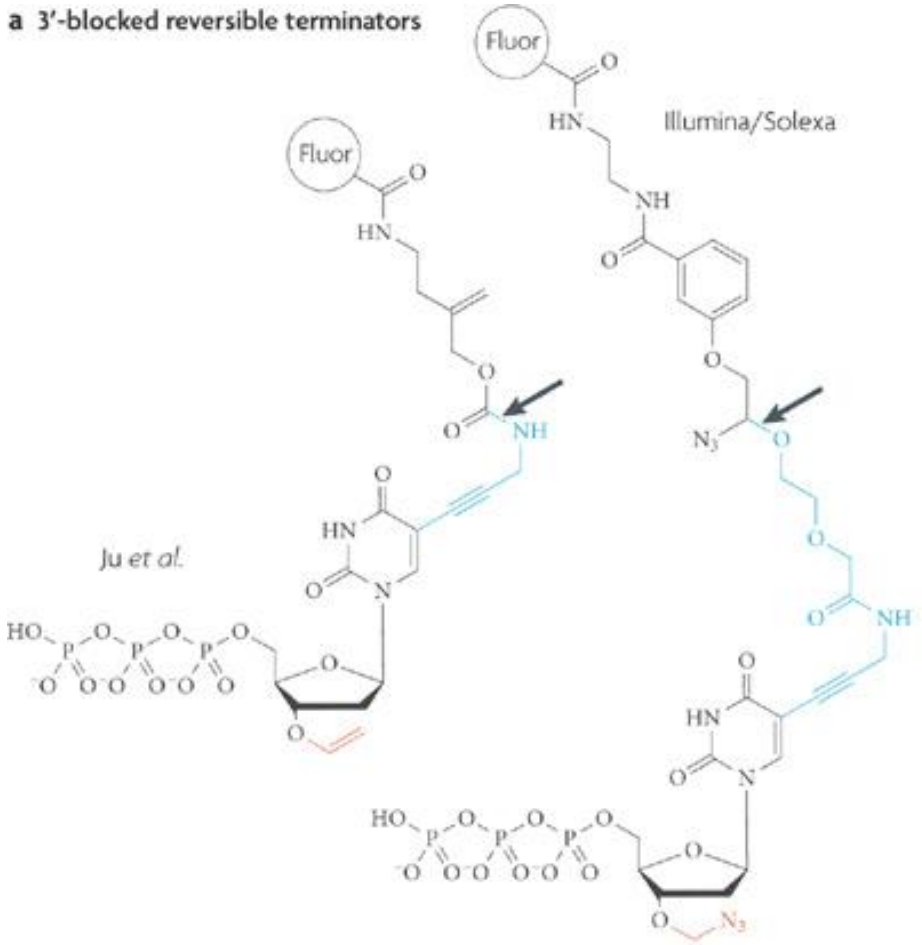
Sequencing-by-synthesis

Separate fluorescent tags on each nucleotide

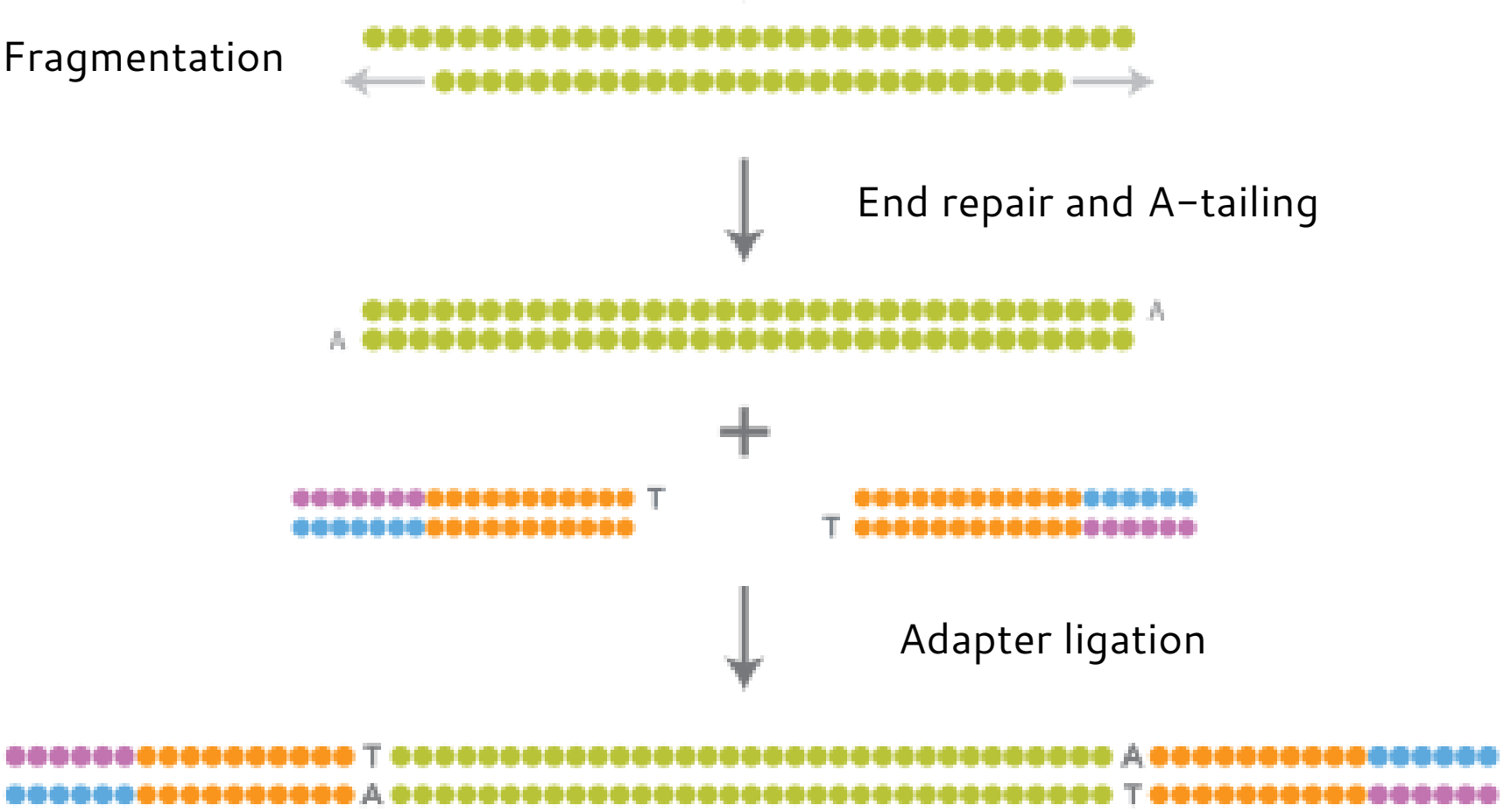
Reversible terminators



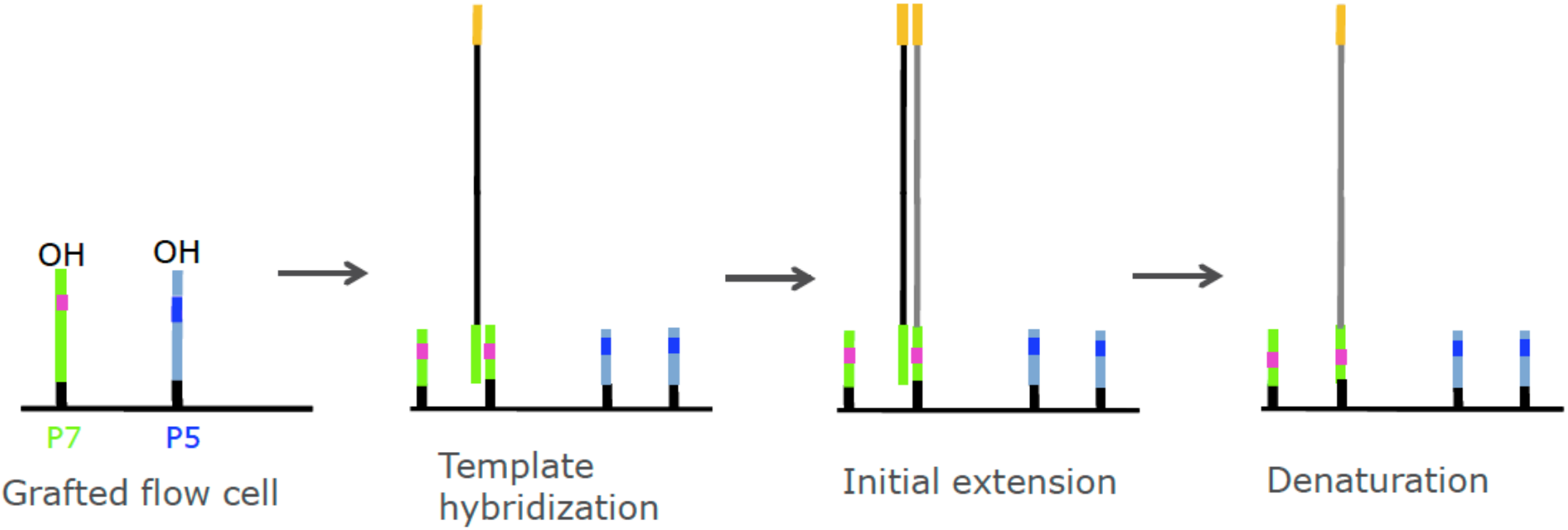
Illumina chemistry – reversible terminators



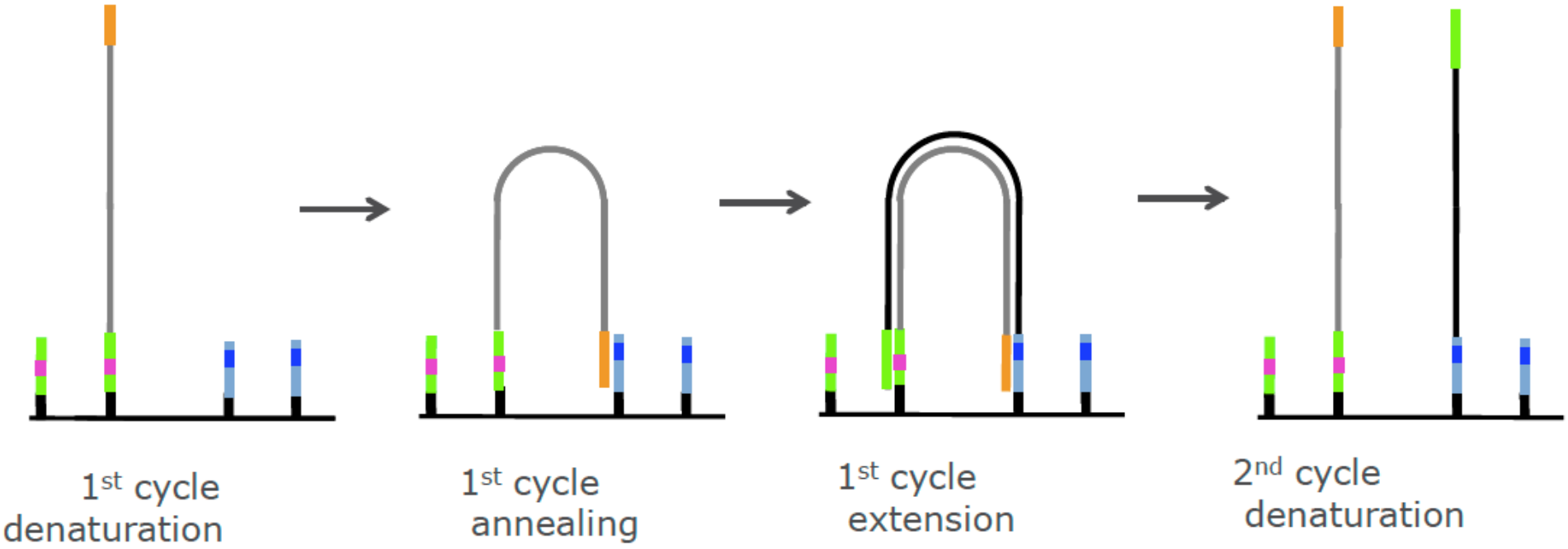
Library preparation steps



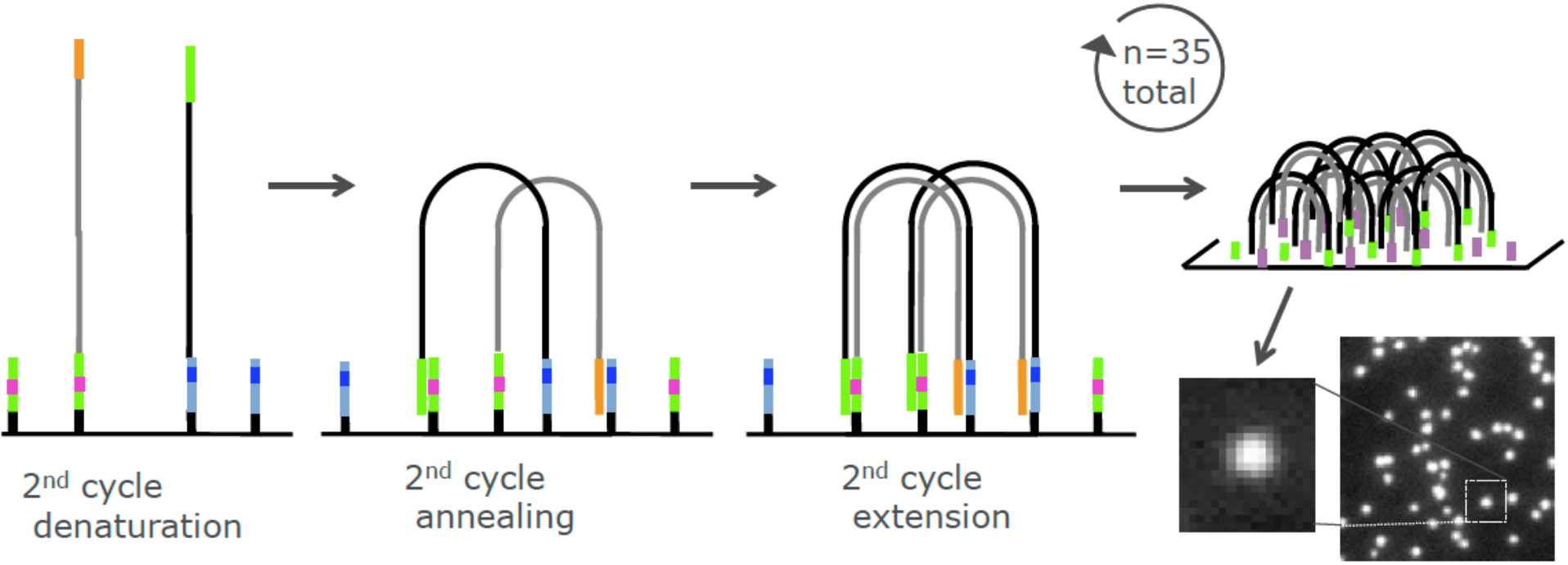
Getting your library on a flowcell



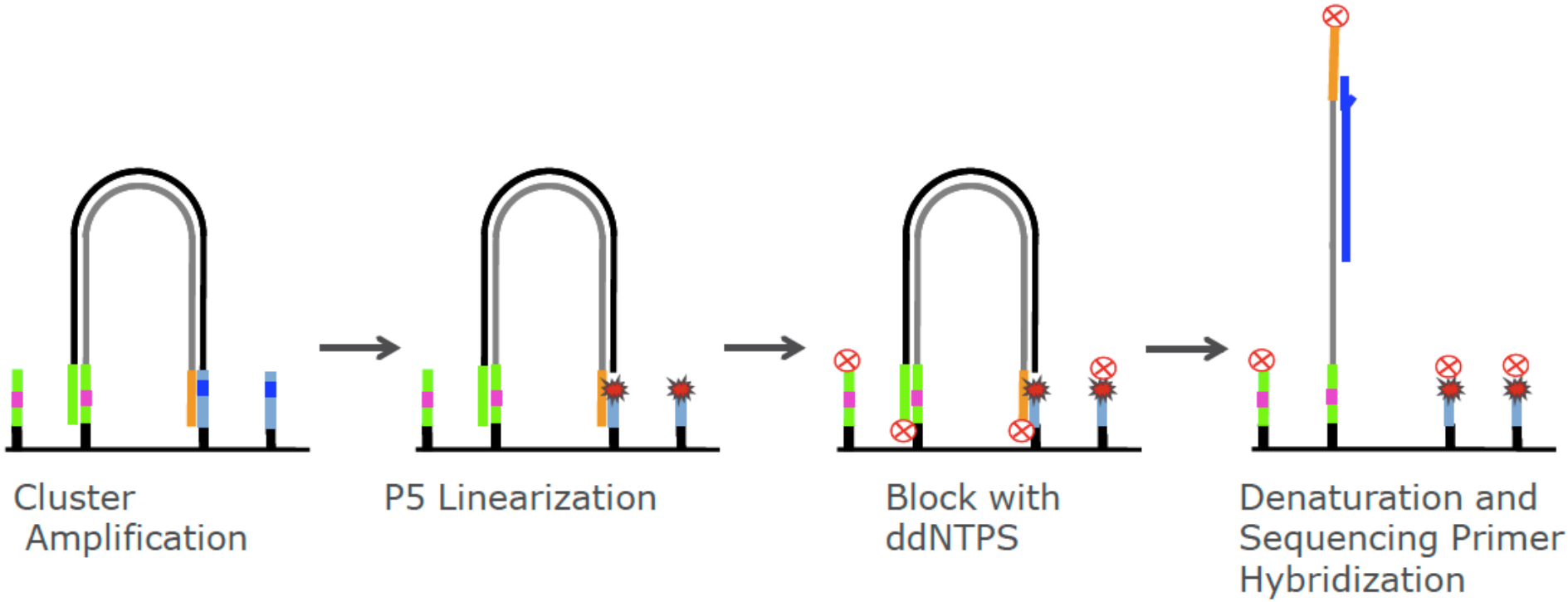
Cluster generation – Bridge amplification



Cluster generation – Bridge amplification

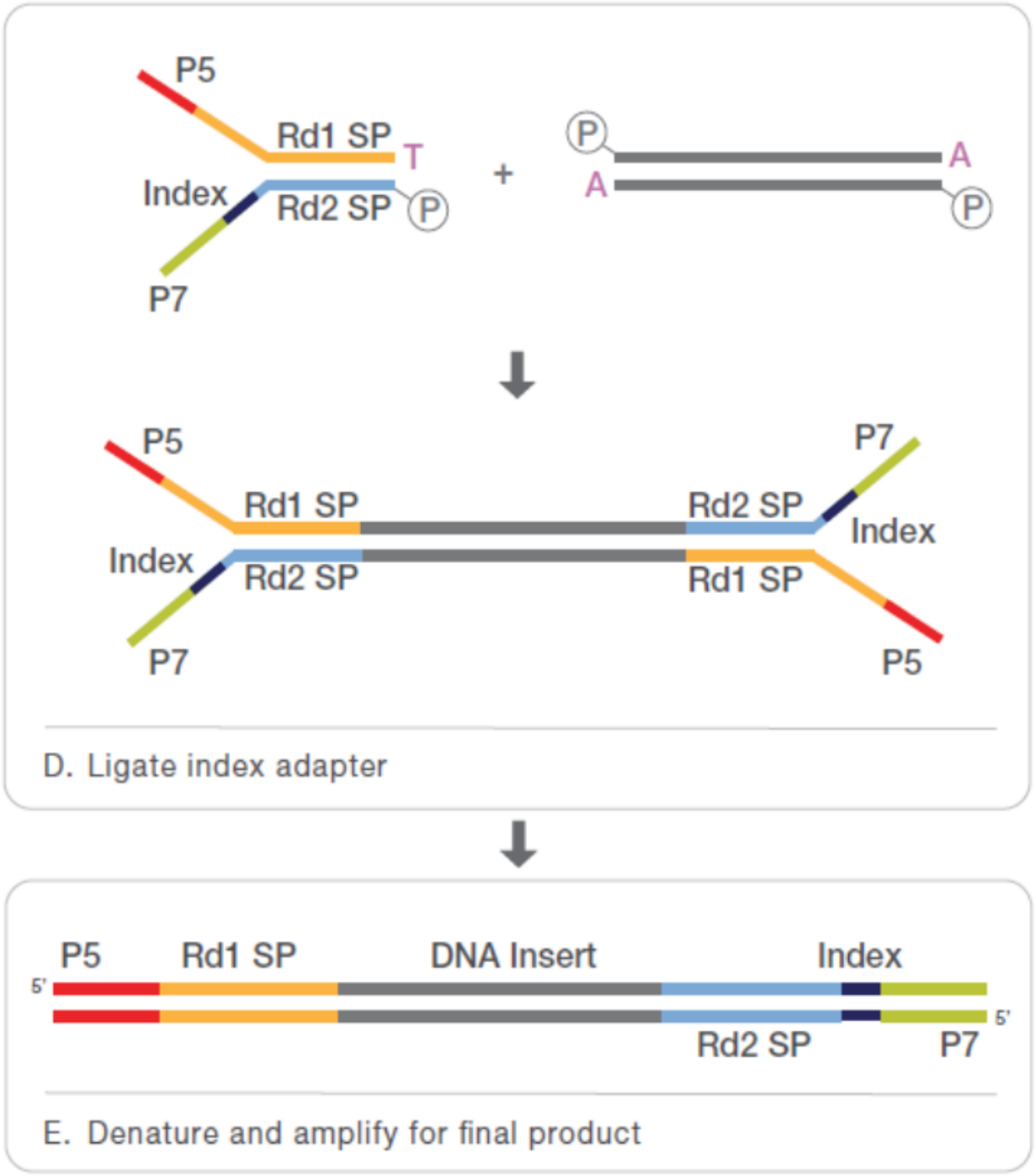


Sequencing



Single end – sequence one end

Paired end – sequence both ends



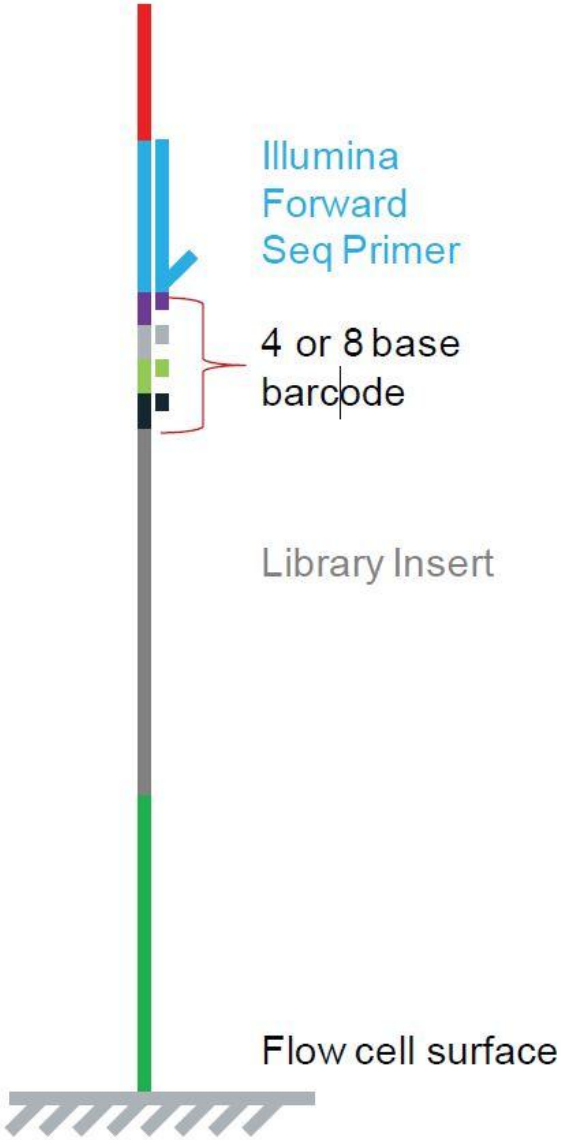
TruSeq Adapters

Forked adapters

Not complete without PCR

Indexes/ barcodes allow for multiplex sequencing using a third sequencing read (currently up to 24)

In-line multiplex adapters



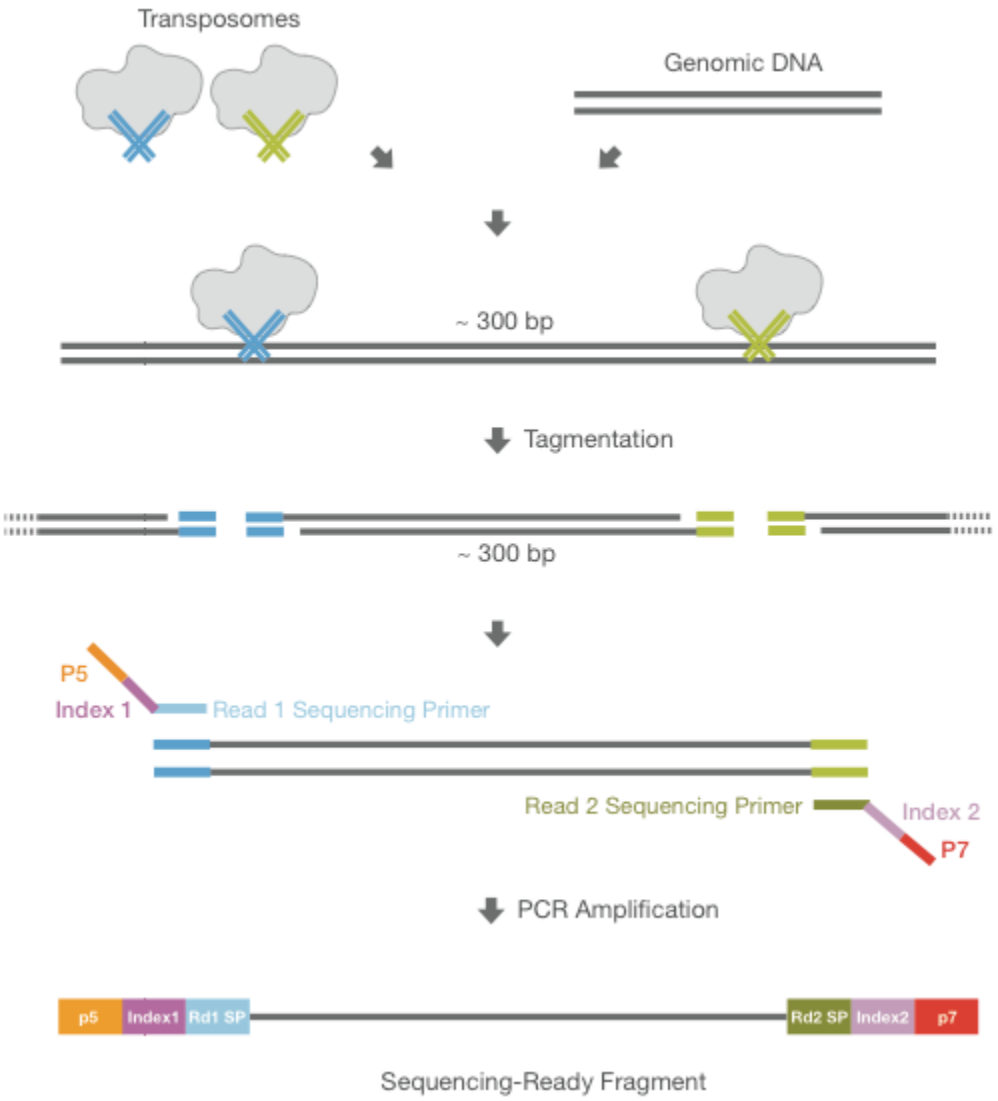
Indexes/barcodes allow for multiplex sequencing

Index/barcode is in the first 4-8 bases of the sequencing read

Allows for more indexes (currently up to 384)

Initial bases MUST be distributed randomly

Nextera "tagmentation"



Uses transposomes to fragment DNA and add adapters simultaneously

Review

- Numerous technologies out there, with more in development.
- Illumina libraries need adapters to anneal properly to the flowcell.
- Illumina technology employs sequencing by synthesis, fluorescence imaging, and microfluidics.

Break

Minute cards!

Why are you taking this course?

What do you want to learn?

What do you already know about RNA-Seq?

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- Cluster test
- Sequencing technologies
- **Planning an RNA-seq experiment**
- **Library prep**
- Design exercises
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ENCODE
(Encyclopedia of DNA elements)

"The goal of ENCODE is to build a comprehensive parts list of functional elements in the human genome, including elements that act at the protein and RNA levels, and regulatory elements that control cells and circumstances in which a gene is active."

30 papers published
simultaneously in September

ENCODE

Standards, Guidelines and
Best Practices for RNA-Seq

Detailed document describing desirable characteristics of an RNA-Seq experiment

Not comprehensive (designed for cell lines)

Not necessary to follow all the guidelines, depending on your experiment

Planning an RNA-Seq experiment

- **How will I make the cDNA?**
- How will I remove rRNA, gDNA, and mitochondrial sequences?
- How will I prepare the library?
- How many PCR cycles?
- How much sequencing should I do?
- Should I align or assemble?
- What should I align to?
- How do I quantify expression?
- How do I test for differential expression?

It depends...

Source RNA

Two main concerns:

Amount (best assessed with Ribogreen)

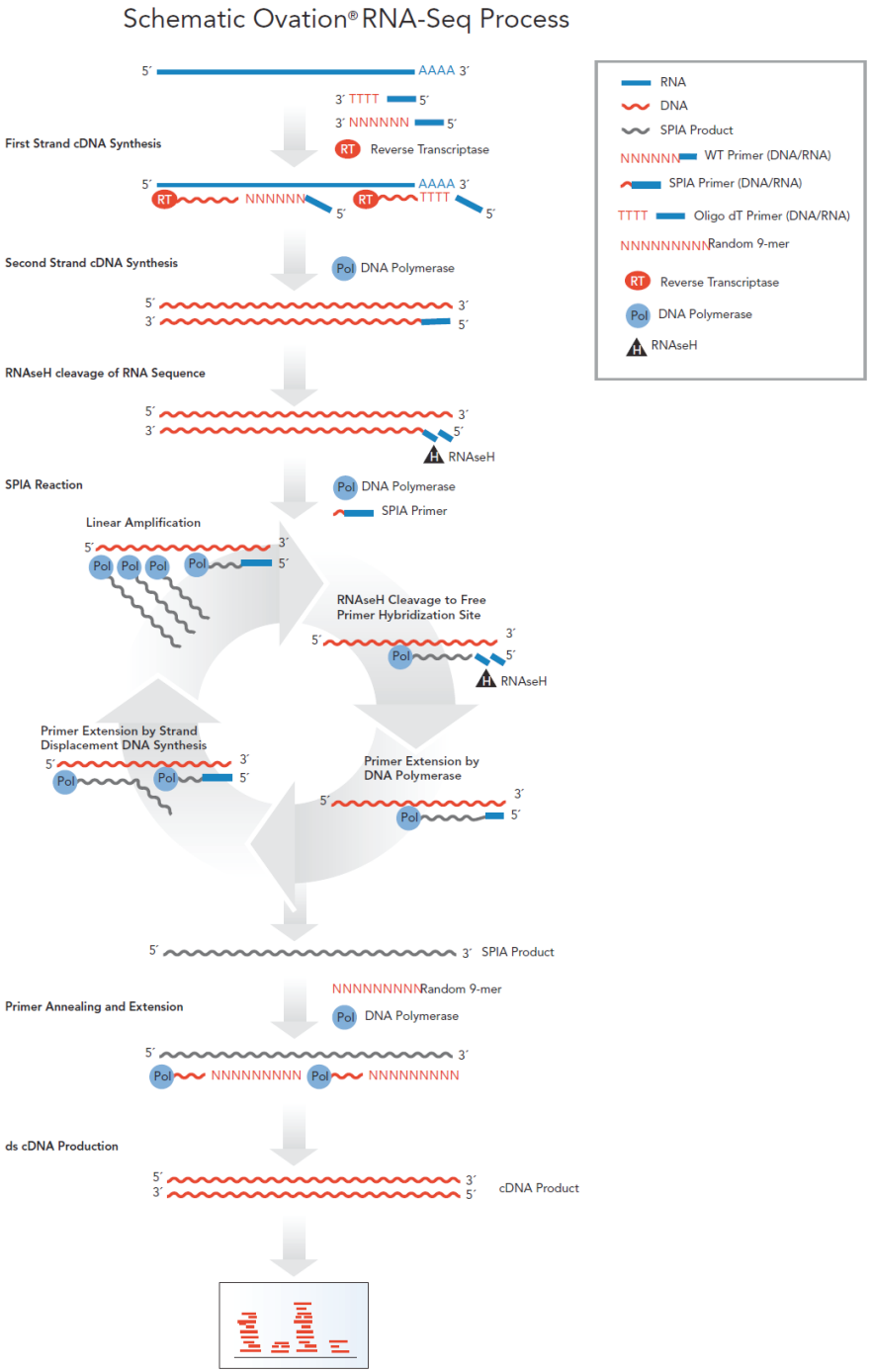
≥100 ng, use any kit

<100 ng, could require amplification

Quality (best assessed with Bioanalyzer)

High RIN, use any kit

Low RIN, RT with random hexamers

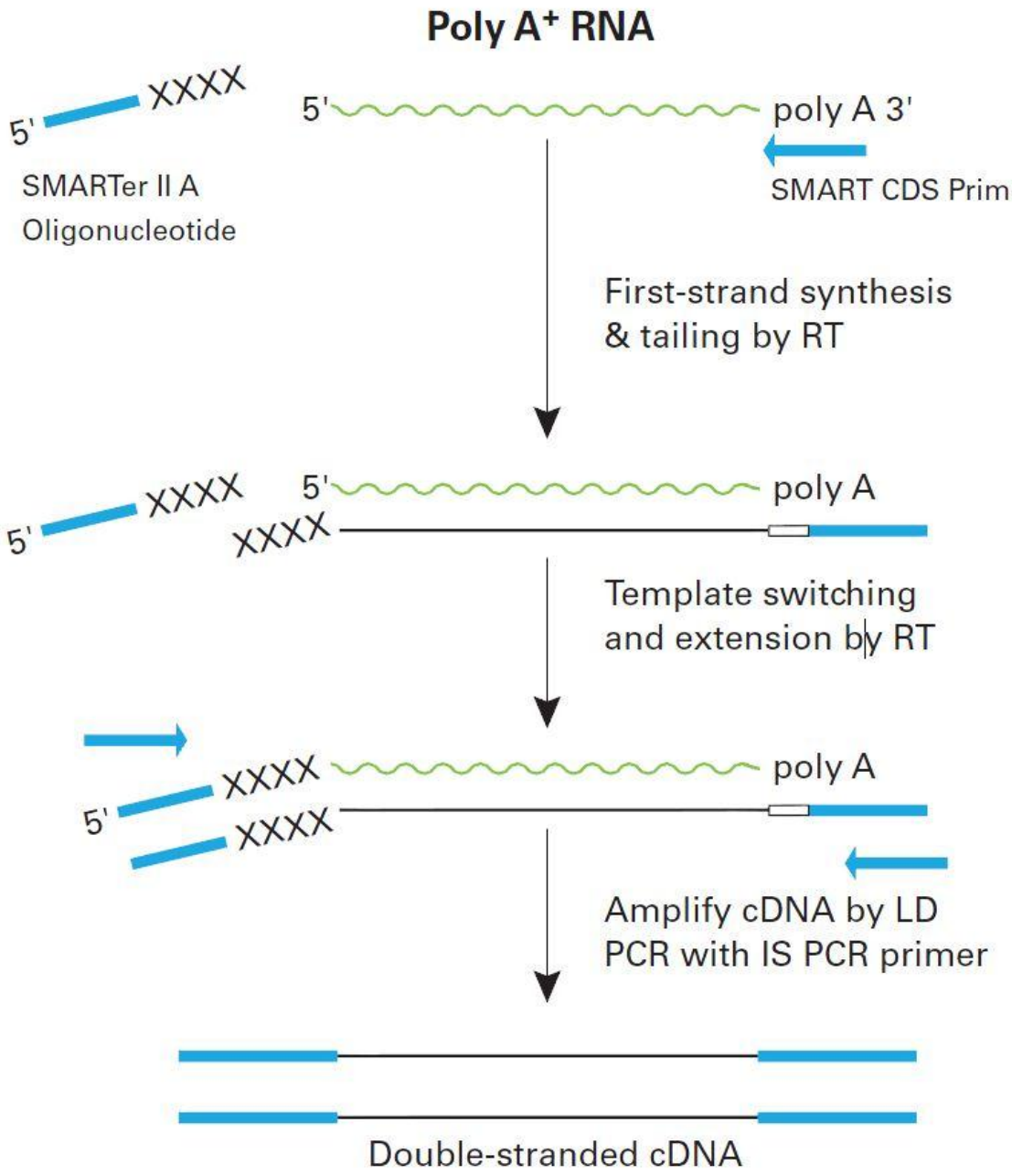


Nugen Ovation

Linear amplification

Works with fragmented samples

Allows sub-nanogram input (hundreds of pgs)



Clontech SMARTer Ultra Low

Requires high quality RNA

Uses oligo dT priming for RT

Allows single cell input (10pg)

Reverse Transcription

Two main options for reverse priming:

Oligo dT

requires high quality RNA

can produce an AT bias and a 3' bias

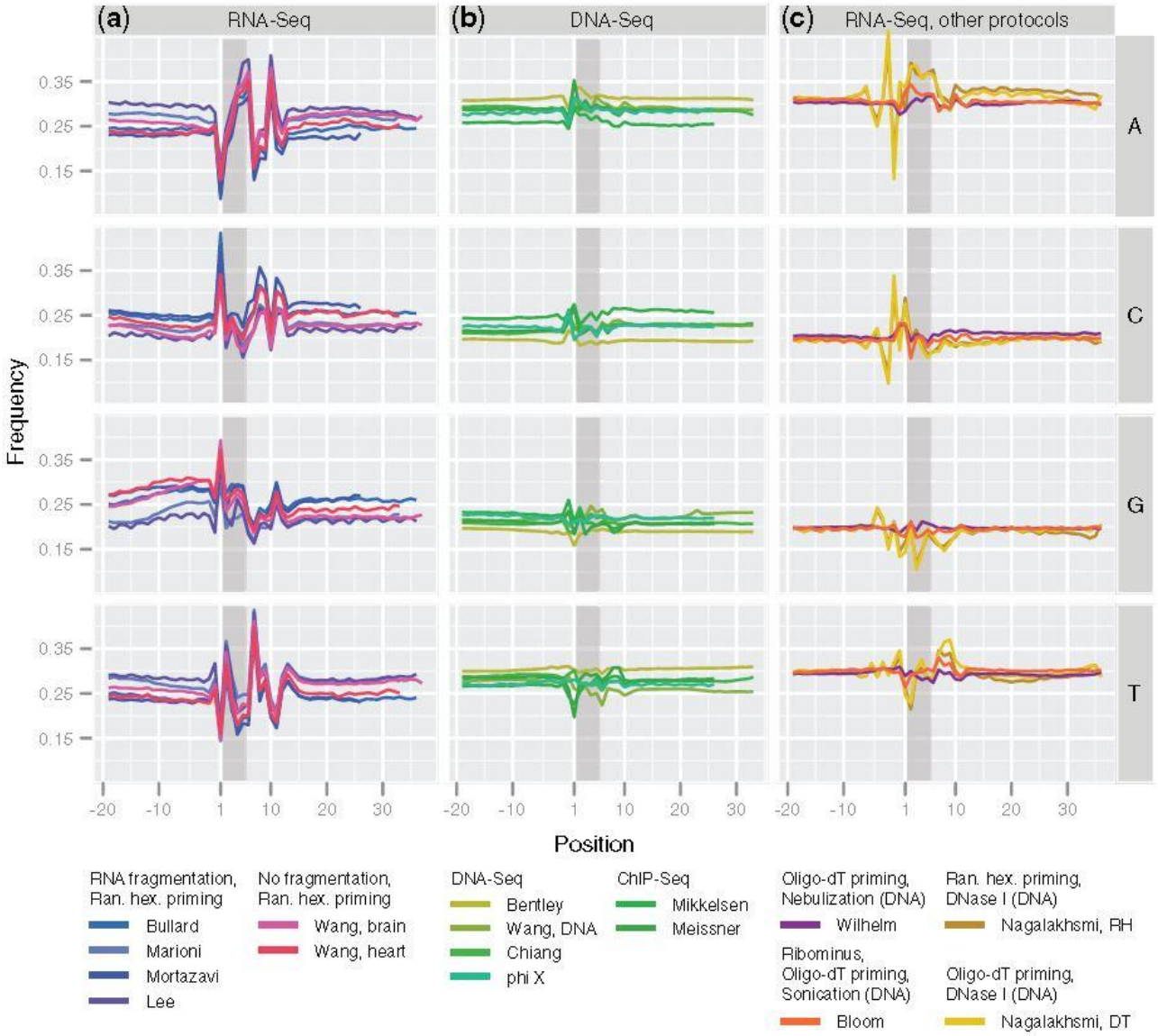
Random primers

works with fragmented RNA

not really random; produces a bias at the beginning of the read

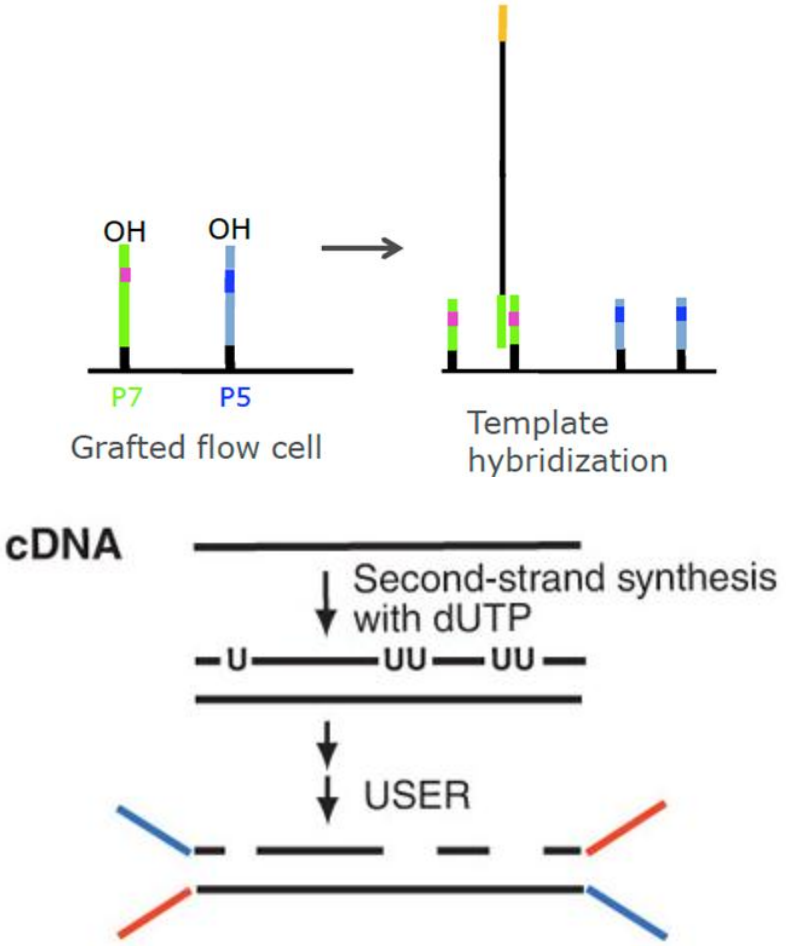
Use a mixture of both

Random Hexamer bias



Hansen, K.D., Brenner, S.E., and Dudoit, S. (2010). Nucleic Acids Research *38, e131.*

Strand specific sequencing



Standard RNA-Seq does not give strand information

Need a separate step to distinguish between strands

Useful for more complicated genomes with overlapping genes or for discovery

Levin, J.Z., et.al.(2010). Comprehensive comparative analysis of strand-specific RNA sequencing methods. Nat Meth

Planning an RNA-Seq experiment

- How will I make the cDNA?
- **How will I remove rRNA, gDNA, and mitochondrial sequences?**
- How will I prepare the library?
- How many PCR cycles?
- How much sequencing should I do?
- Should I align or assemble?
- What should I align to?
- How do I quantify expression?
- How do I test for differential expression?

Removing genomic DNA and mtDNA

DNase treatment if at all possible

RNA-Seq is a **sampling experiment**!

The more unwanted sequences you have in your sample, the fewer useful reads you will get in the end.

Removing ribosomal RNA

rRNA is >90% of the RNA in a cell!

polyA selection

Collect polyadenylated RNAs

oligo dT beads

requires high quality RNA

can introduce 3'bias, AT bias

no organisms without polyadenylation

Removing ribosomal RNA

Subtractive hybridization

- Pull out rRNA with specific probes
- Preserves non-polyadenylated RNA
- Products not available for all species
- Best choice for bacteria

Removing ribosomal RNA

Not so random primers

- Primers for RT are screened against rRNA sequences
- Works for polyA and non-polyA
- Increased sequence bias

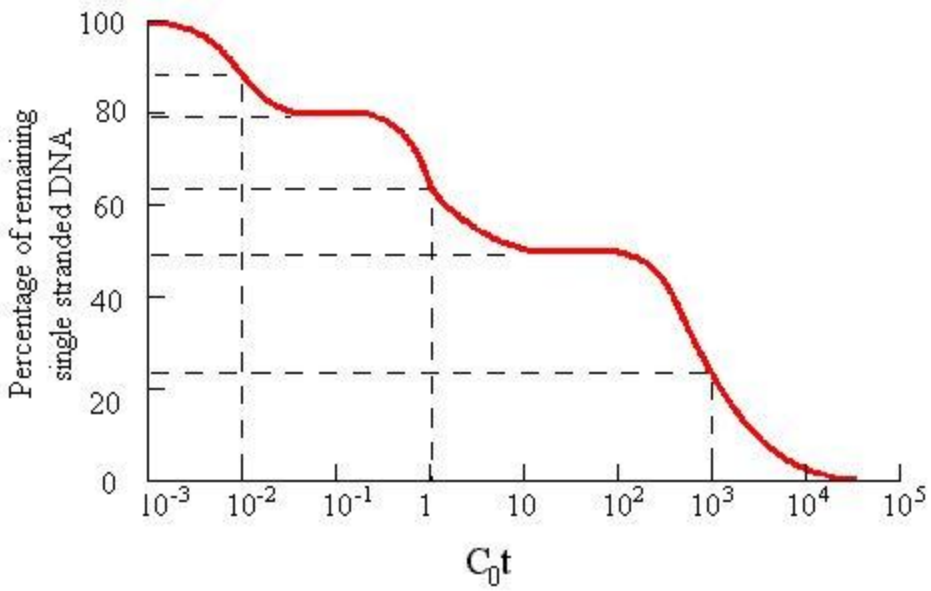
Removing ribosomal RNA

DSN (double strand nuclease) normalization

Very tricky to optimize

Not for DE

Good choice mainly for organisms with no reference



Removing ribosomal RNA

Selective hybridization/PCR amplicons

- Select known target genes
- You pick what you want to sequence!
- Not for discovery
- Thoroughly developed hypothesis
- Can be expensive
- Need to be careful about low complexity

Planning an RNA-Seq experiment

- How will I make the cDNA?
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- **How will I prepare the library?**
- How many PCR cycles?
- How much sequencing should I do?
- Should I align or assemble?
- What should I align to?
- How do I quantify expression?
- How do I test for differential expression?

RNA or cDNA fragmentation

Fragmentation must occur at some point

The larger the insert, the
lower the clustering efficiency

100–300 bp insert size is best

Fragment size = size of insert + adapters (120bp)

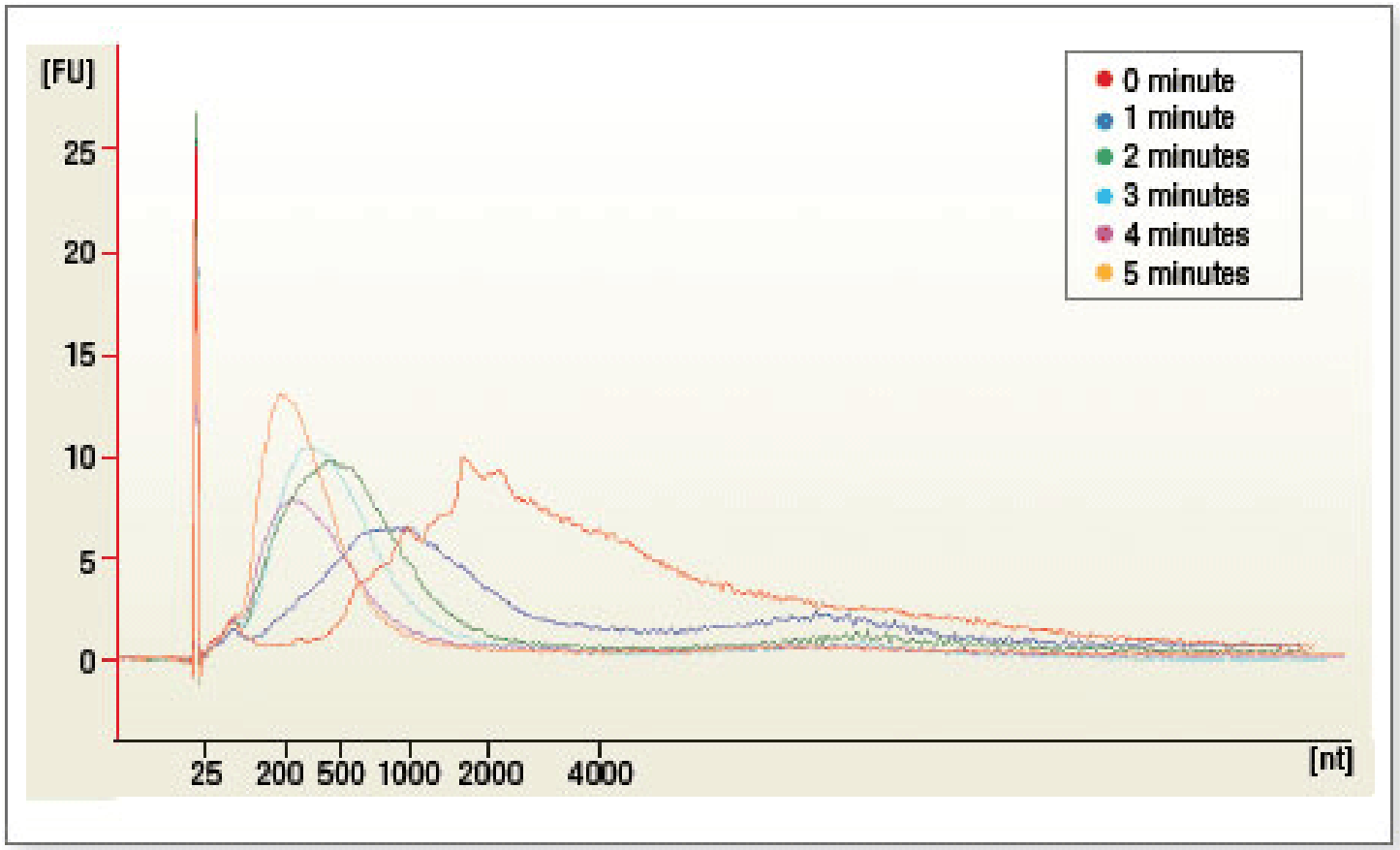
Insert size = size of insert without adapters

Mate inner distance = size of unsequenced portion of insert after paired end sequencing

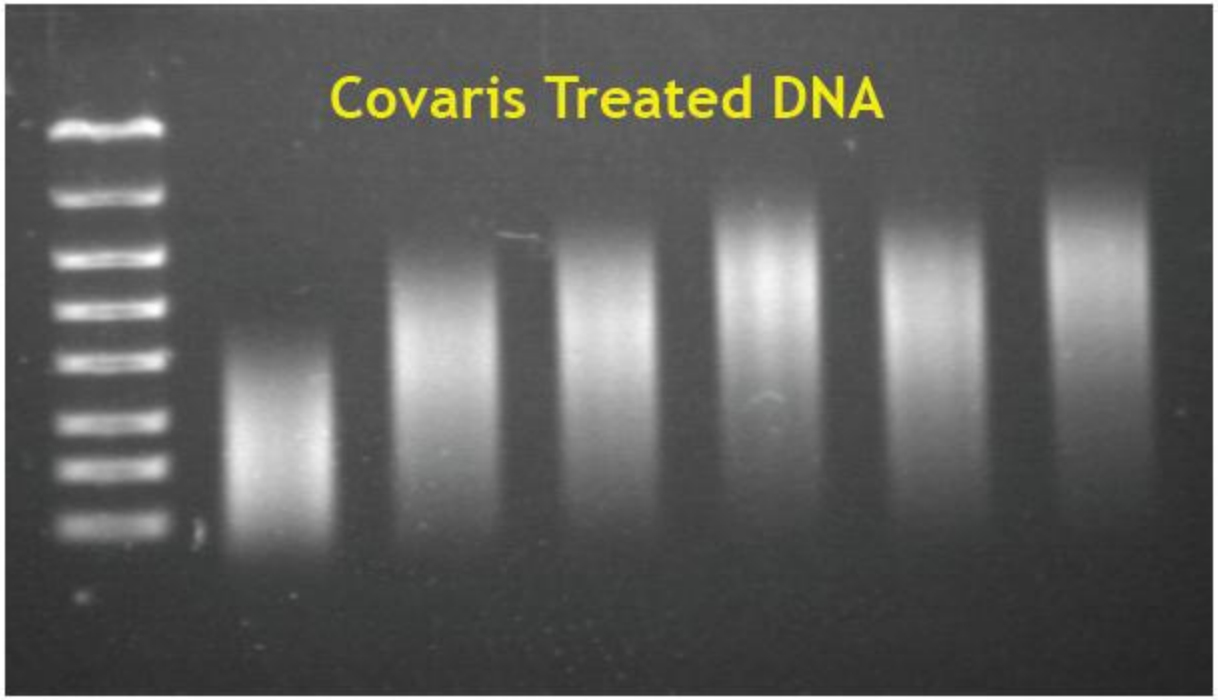
Fragment size – adapters – (2 X read length)

RNA fragmentation

Fragment RNA with Mg^{2+} and heat



cDNA fragmentation



Options for cDNA fragmentation

- Nebulization
- Sonicators (**Bioruptor**, Covaris)
- Enzymatic (can introduce bias)
- Transposon (Nextera)

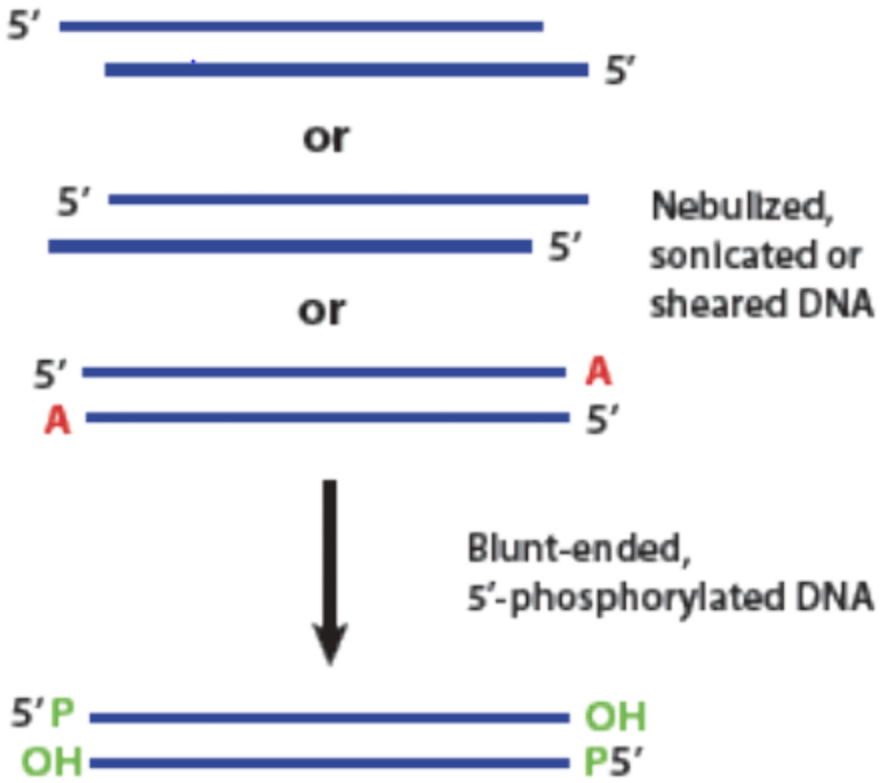
End Repair

Fragmented ends ligate poorly

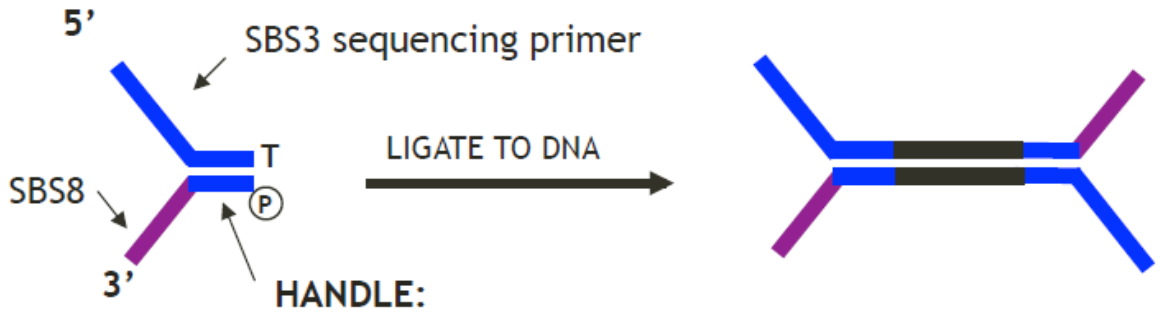
Blunt the ends

(Klenow DNA polymerase, T4 DNA polymerase, T4 PNK)

A-tailing



Adapter Ligation



HANDLE:
13 base complement of the 3' end of the SBS3 top strand

Ligate to A-tailed DNA

GAATTCGAGTCTA
ACTTAAGCTCAGA

ACACTCTTTCCCTACACGA CGCTCTTCCGATCT GAATTCGAGTCTA GATCGGAAGAGC GTTCAGCAGGAATGCCGAG
GCGAGAAGGCTAG ACTTAAGCTCAGA TCTAGCCTTCTCG
GAGCCGTAAGGACGACTTG CAGCACATCCCTTTCTCACA

PCR w Primer 1.0 and Primer 2.0

Adapter ligation

Truseq LT

- TS Univ. Adapter: AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT
- TS Index Adapter: GATCGGAAGAGCACACGTCTGAACTCCAGTCAC[I]ATCTCGTATGCCGTCTTCTGCTTG
- Primer P1: AATGATACGGCGACCACCGA
- Primer P2: CAAGCAGAAGACGGCATACGA
- Multiplexing Read 1 Seq Primer: ACACTCTTTCCCTACACGACGCTCTTCCGATCT
- Multiplexing Index Read Seq Primer: GATCGGAAGAGCACACGTCTGAACTCCAGTCAC
- Multiplexing Read 2 Seq Primer: GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT

Seq: ACACTCTTTCCCTACACGACGCTCTTCCGATCT GATCGGAAGAGCACACGTCTGAACTCCAGTCAC

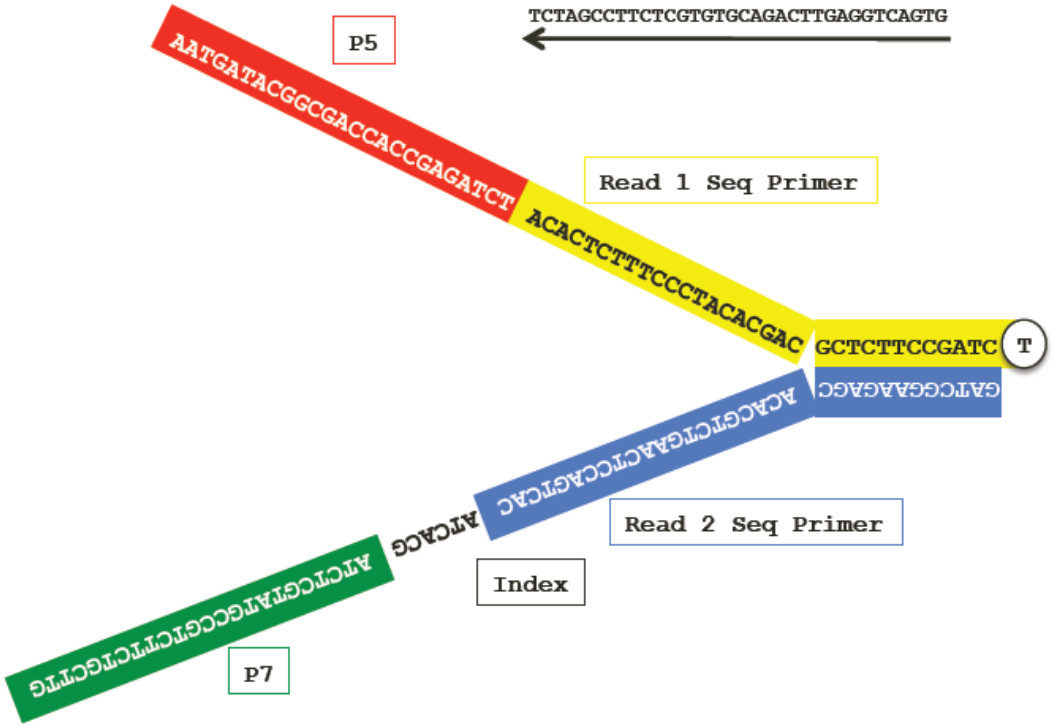
PCR: AATGATACGGCGACCACCGA

AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-insert-aGATCGGAAGAGCACACGTCTGAACTCCAGTCAC[I]ATCTCGTATGCCGTCTTCTGCTTG

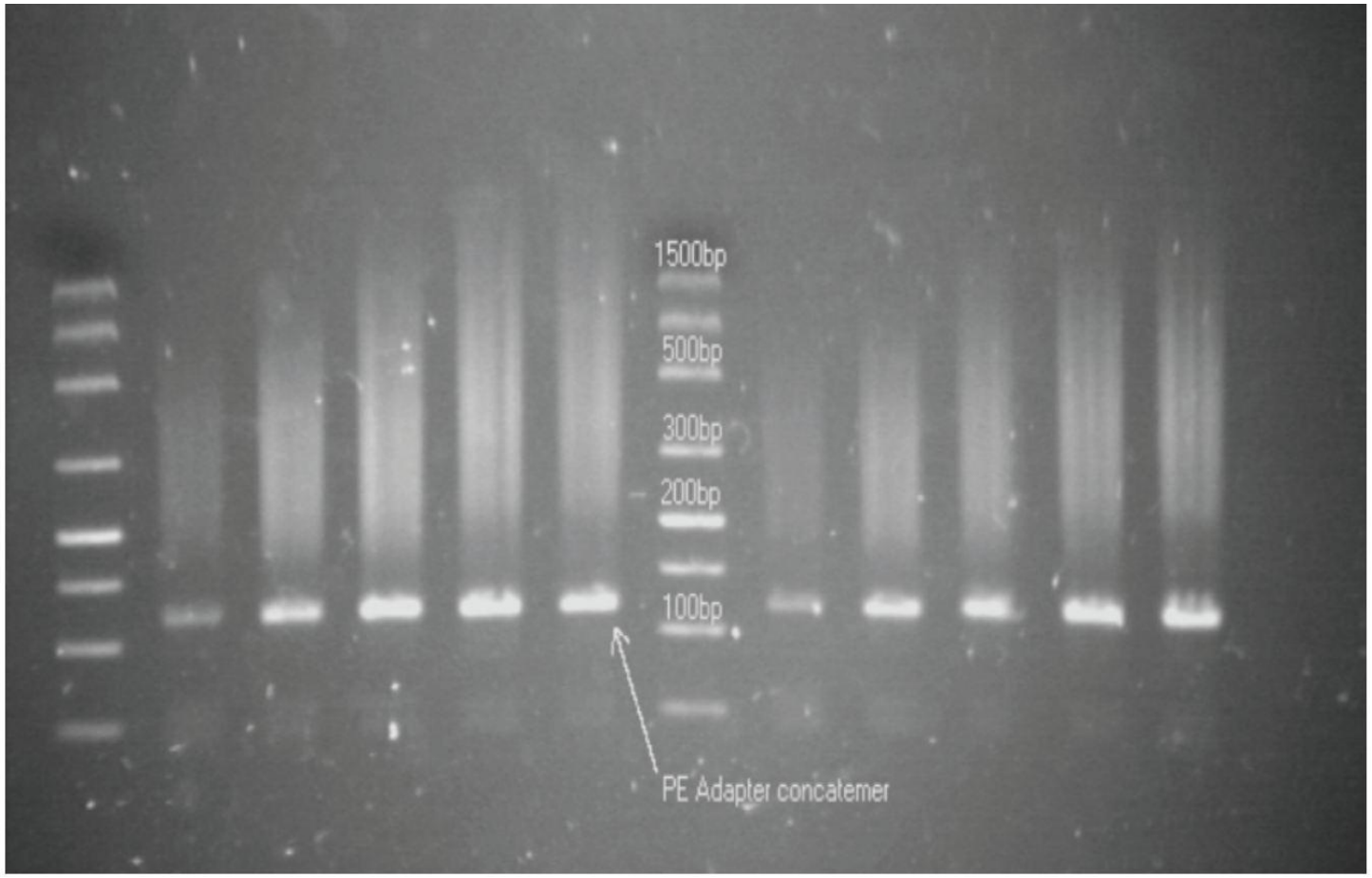
TTACTATGCCGCTGGTGGCTCTAGATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGa-insert-TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG[I]TAGAGCATACGGCAGAAGACGAAC

PCR: AGCATACGGCAGAAGACGAAC

Seq: TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG

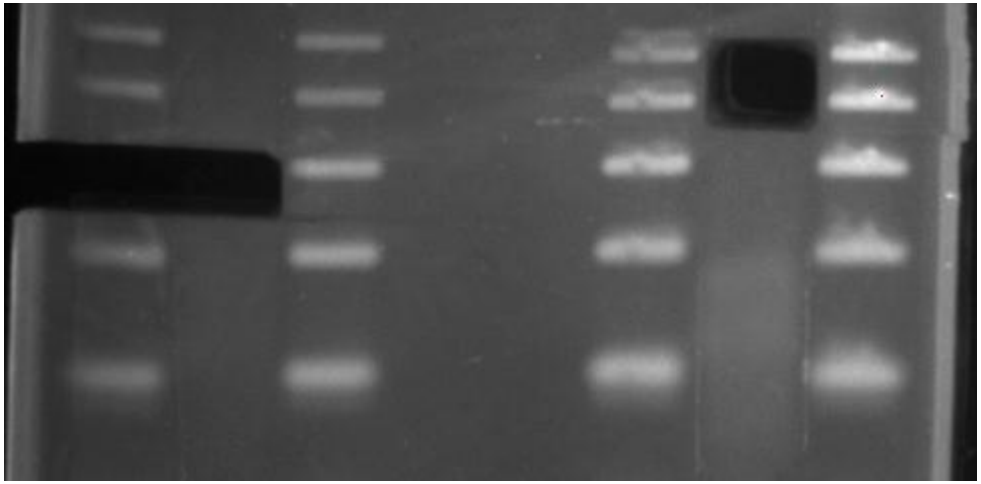


Add the ligase last!



Size Selection

Gel electrophoresis
(Agarose or Acrylamide)



Flexible

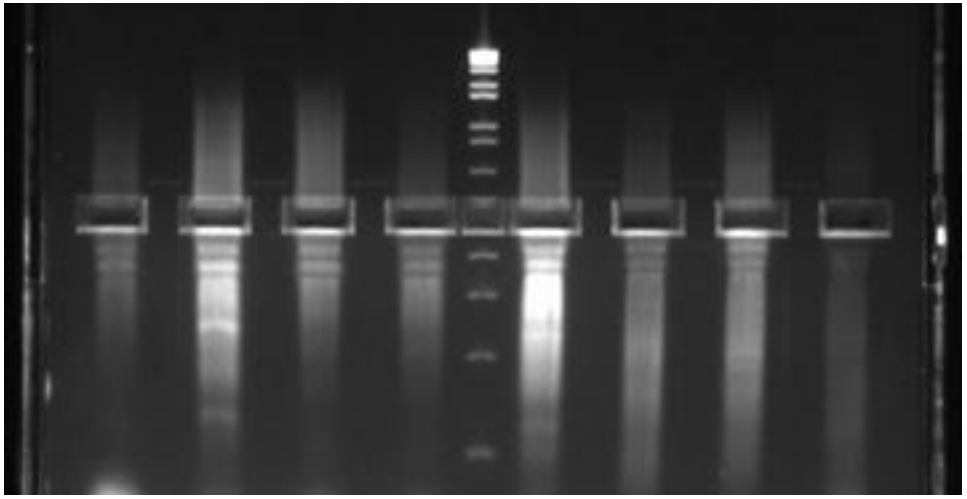
Prone to contamination

Not high throughput

High sample loss (don't heat the sample!)

Easier agarose size selection

Invitrogen E-gel System

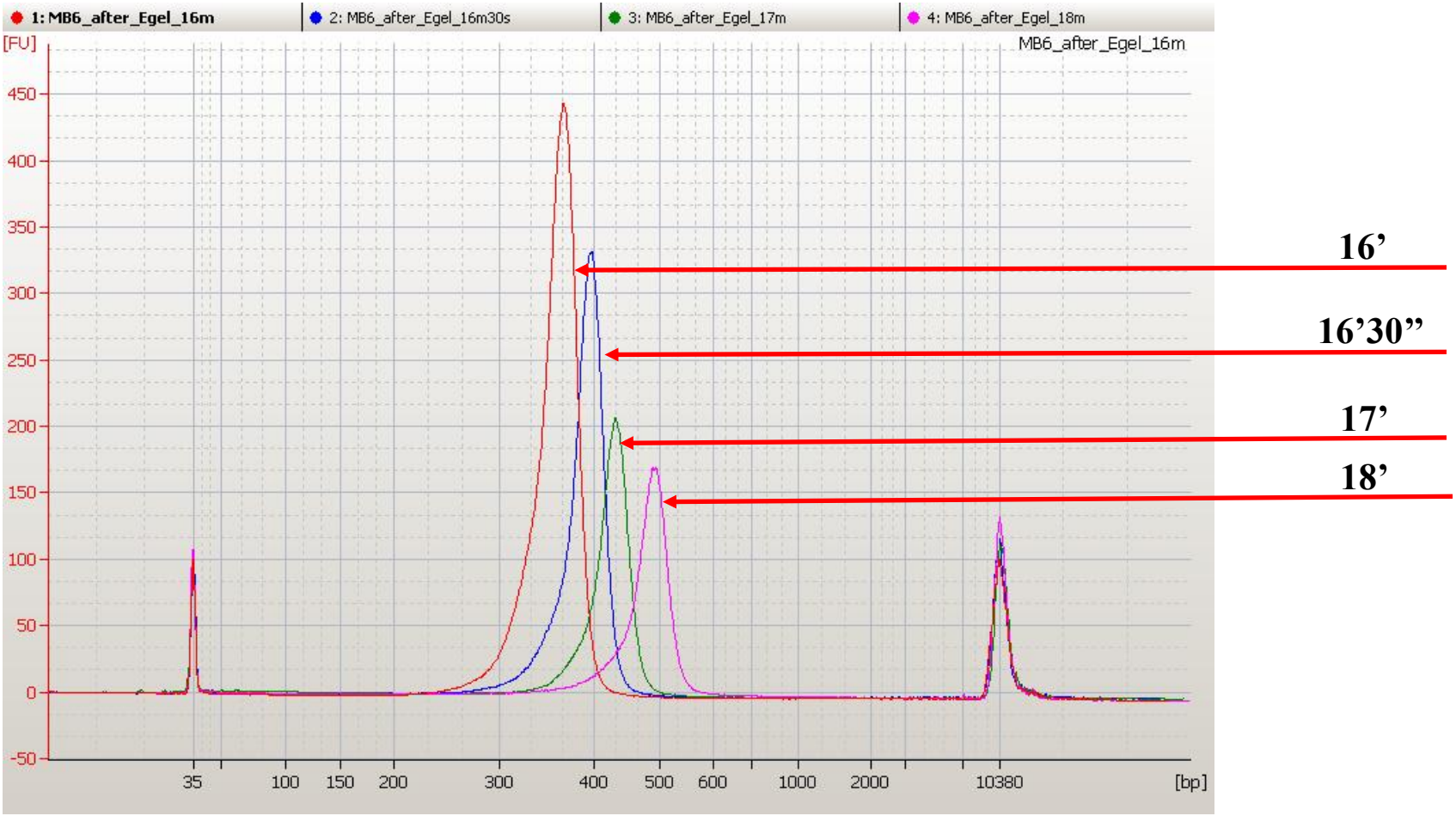


Allows repeated, regular library sizes

Provides downstream flexibility

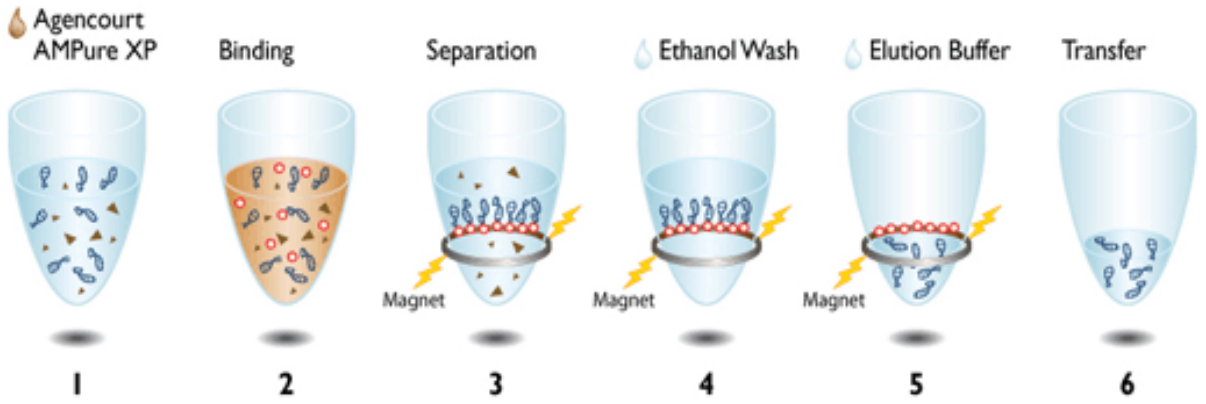
Time consuming

Invitrogen E-gel System



Ampure beads

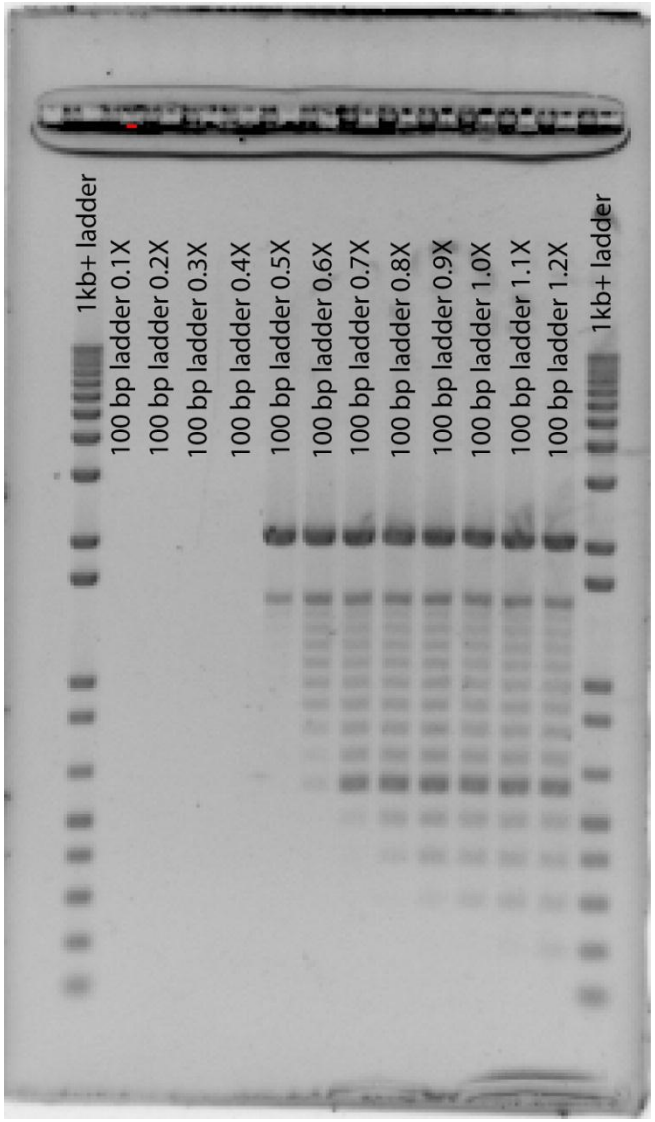
Preferred method of purification of nucleic acids for library preparation



Carboxylate-coated magnetic beads in a PEG and salt buffer

Increasing bead/DNA ratio allows smaller fragments to bind to the beads

Size Selection – Ampure beads

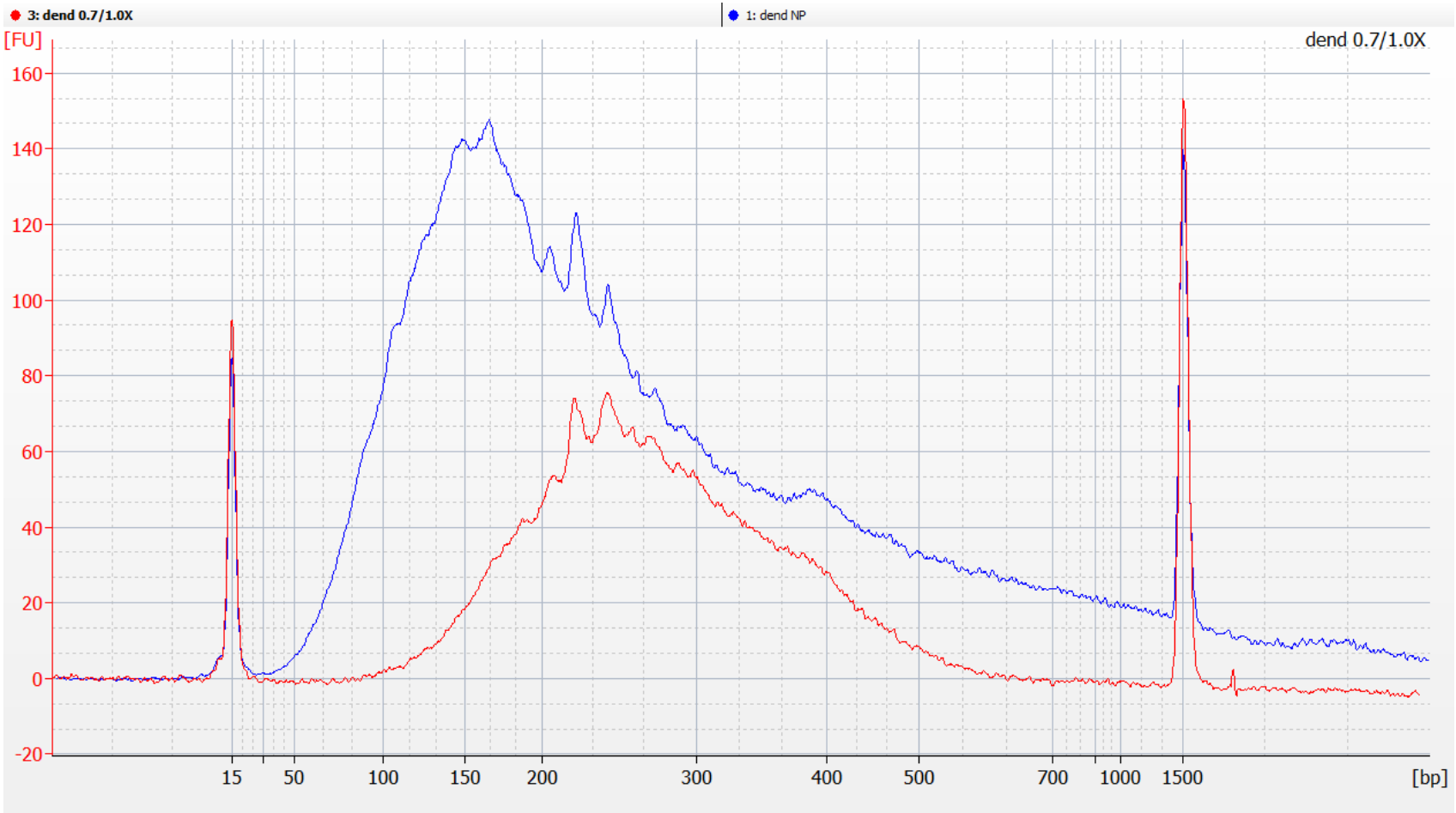


Varying the amount of beads (PEG and salt) relative to the sample allows for different sizes of nucleic acids to be retained

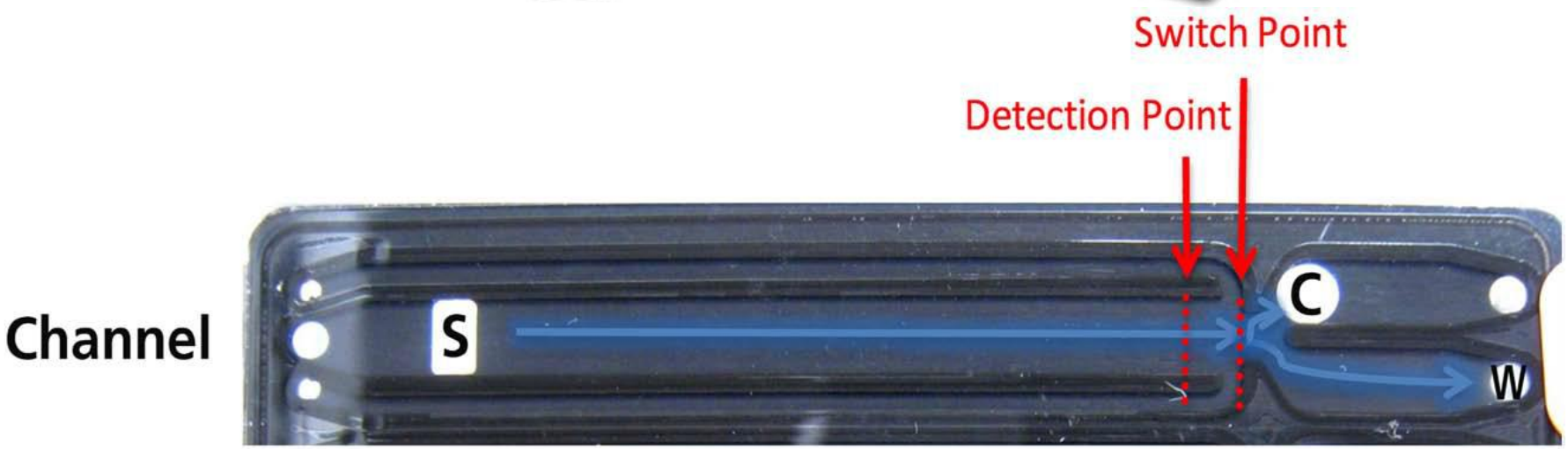
Best for high throughput and automation

Use 1.6-1.8X for purification

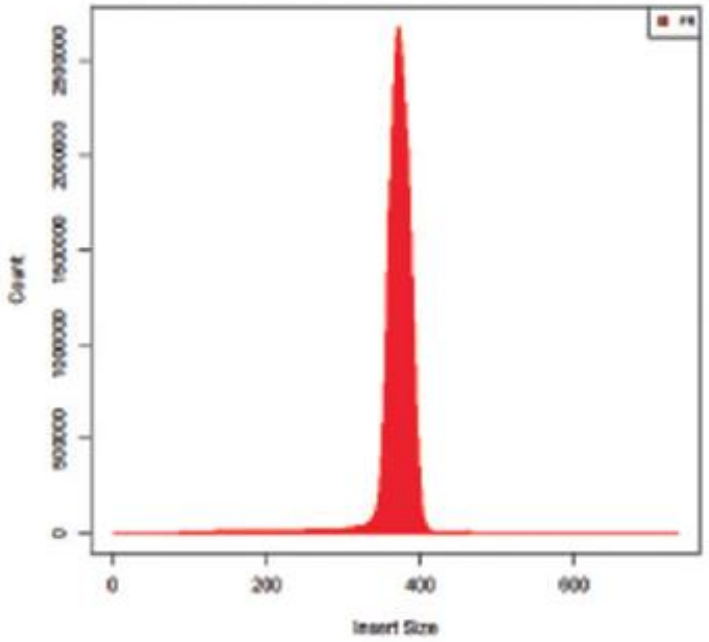
Size Selection – Ampure beads



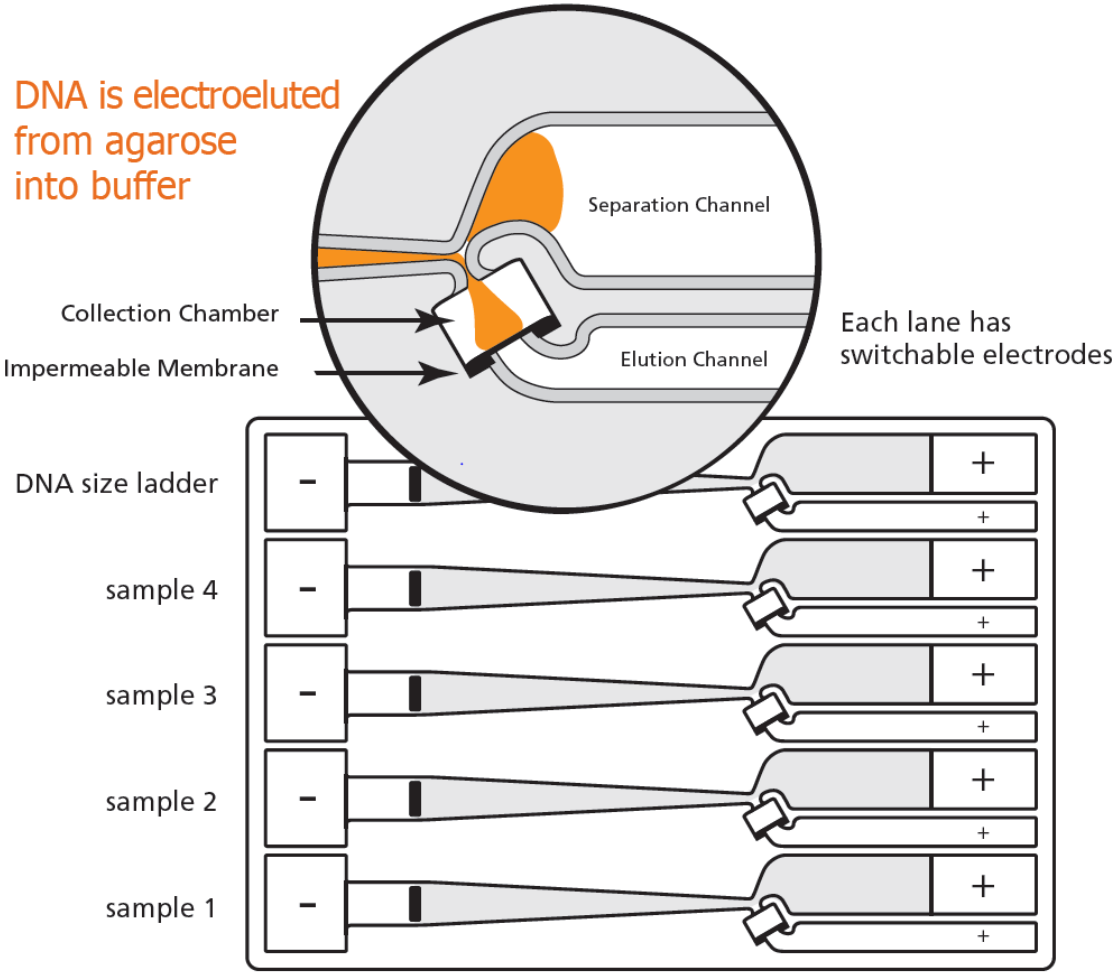
Caliper LabChip XT



Pippin Prep



DNA is electroeluted from agarose into buffer



BluePippin™

Collect targets between 50 bp – 50 kb

FEATURING PULSED-FIELD POWER

Planning an RNA-Seq experiment

- How will I make the cDNA?
- How will I remove rRNA, gDNA, and mitochondrial sequences?
- How will I prepare the library?
- **How many PCR cycles?**
- How much sequencing should I do?
- Should I align or assemble?
- What should I align to?
- How do I quantify expression?
- How do I test for differential expression?

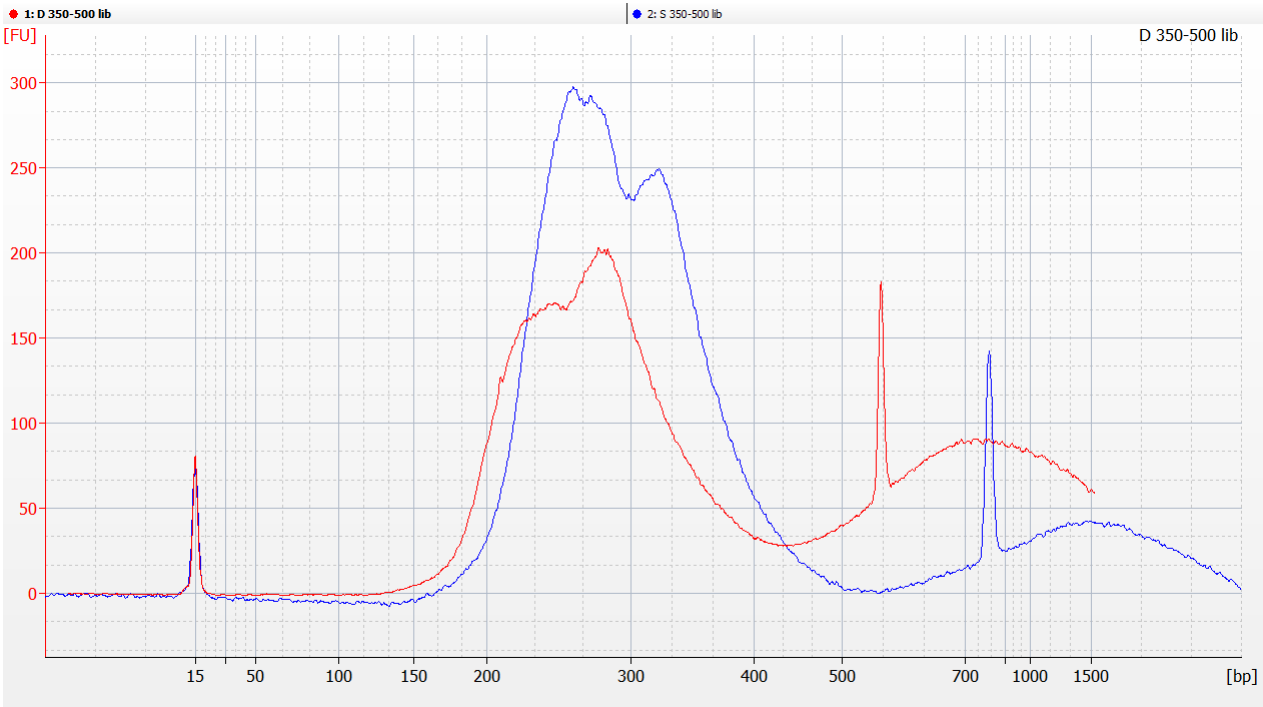
How many PCR cycles?

Goal is to minimize PCR bias

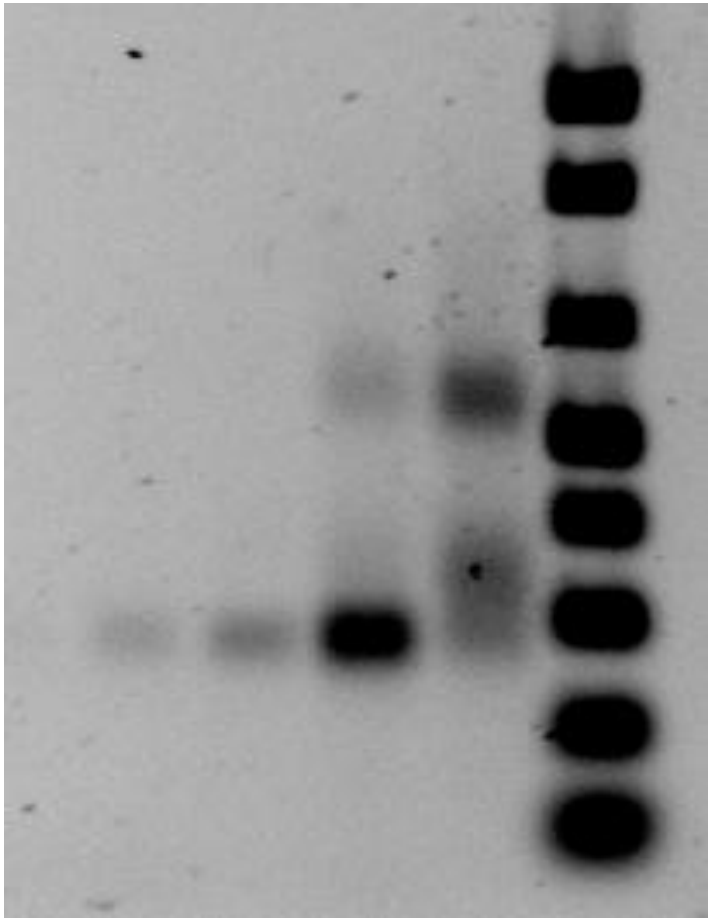
Only one cycle with large input (5ug RNA)

Over-amplification causes various biases

Large product from over-amplification



Optimize your PCR cycles



Manual

Perform 6 cycles of PCR

remove aliquot

Perform 2 cycles of PCR

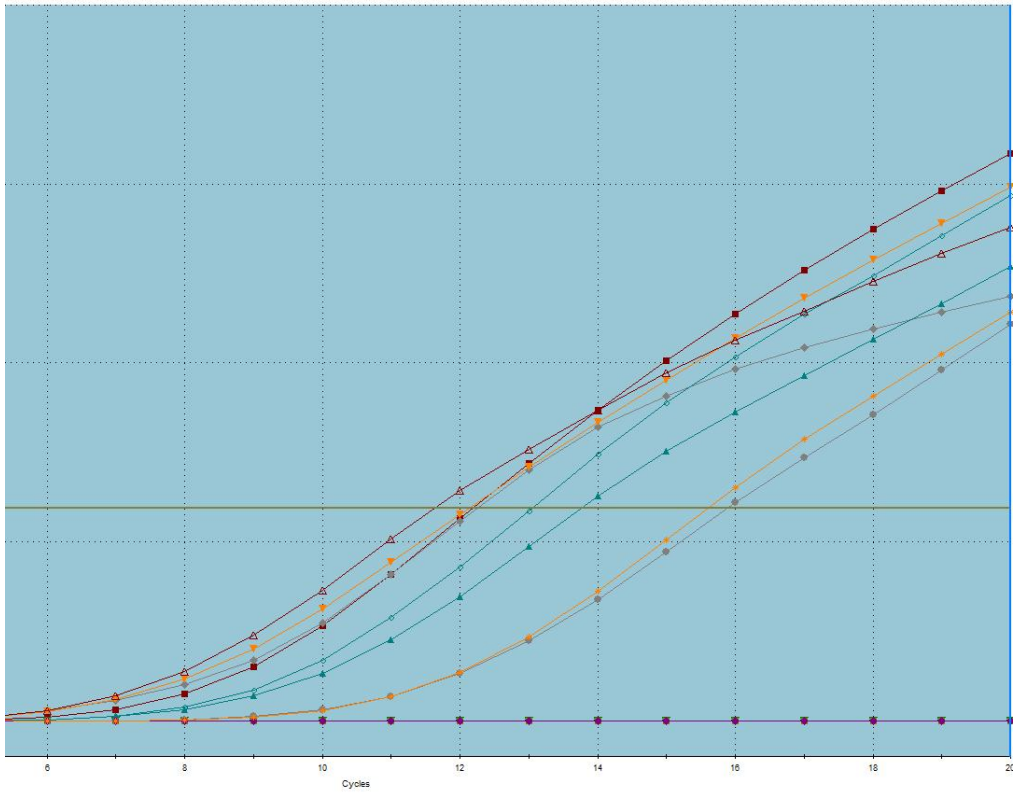
remove aliquot

...

Repeat for 18 total cycles

Agarose gel

Optimize your PCR cycles



qPCR

Determine number of cycles to reach 50% amplification

For RNA-Seq, use that cycle number – 0 or 1

Set up multiple low cycle reactions, pool products

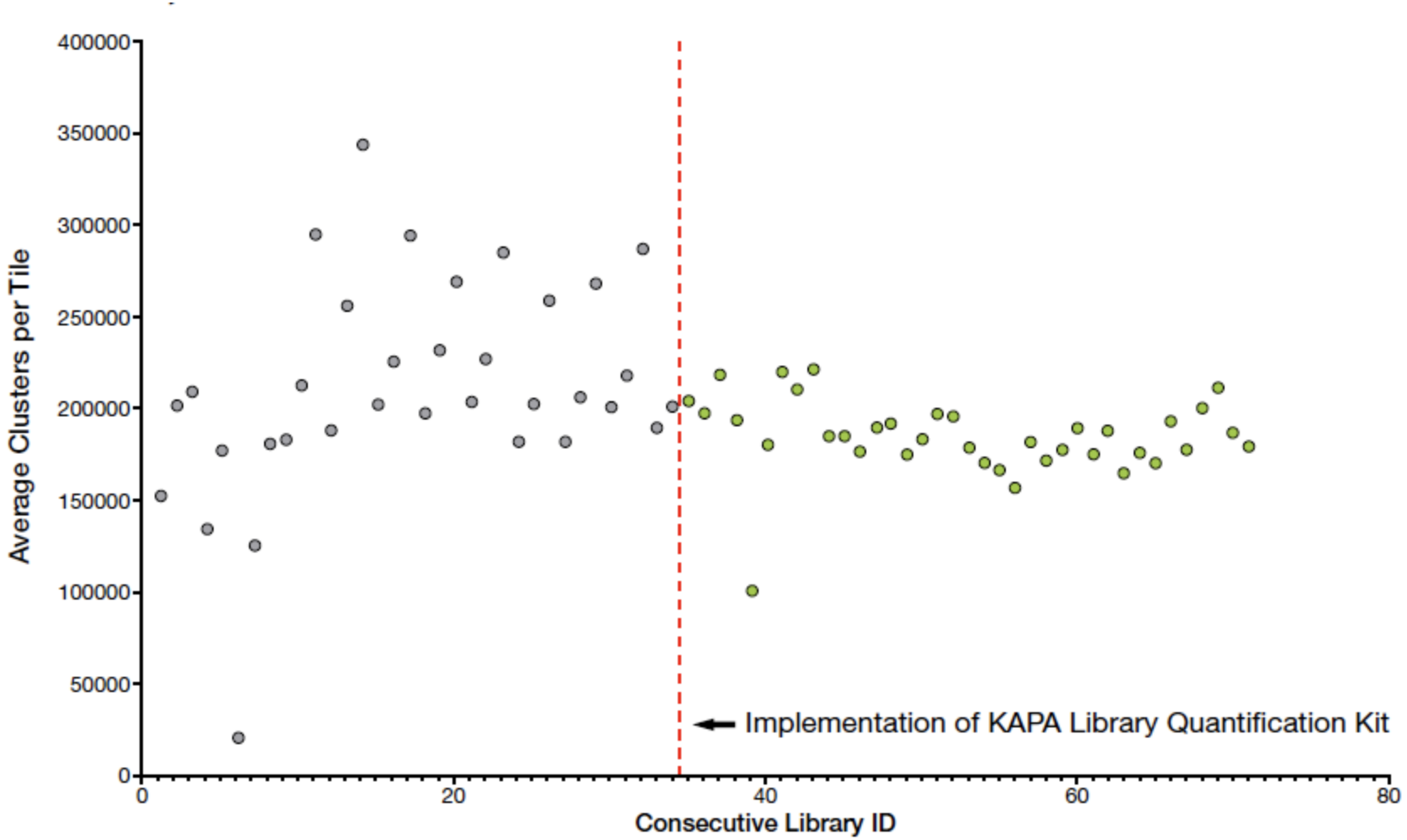
Library Quantitation

In order of increasing accuracy:

1. Nanodrop (UV spectrophotometry)
2. Bioanalyzer
3. Fluorescent Dye (PicoGreen, Qubit)
4. qPCR(KAPA Kit)

Ask your sequencing provider.

KAPA Library Quantification Kit



Minimizing technical variation

Minimize technical variation to reduce sequencing requirements

- Order all reagents needed for the experiment to ensure consistency
- Low retention, filter tip pipettes
- Low binding tubes
- Perform the library prep for all samples simultaneously or follow a randomized block design

Planning an RNA-Seq experiment

- How will I make the cDNA?
- How will I remove rRNA, gDNA, and mitochondrial sequences?
- How will I prepare the library?
- How many PCR cycles?
- **How much sequencing should I do?**
- Should I align or assemble?
- What should I align to?
- How do I quantify expression?
- How do I test for differential expression?

How much sequencing should I do?

As much as you can afford

Proper planning is crucial!

How much sequencing should I do?

What is your question?

What is your genome like?

Prokaryotic or eukaryotic?

Level of alternative splicing?

How much should you budget?

Lane Type	Tufts / Tufts Medical Center	All Other Institutions
Single Read, 50 Base	$1,150	$1,350
Single Read, 100 Base	$1,590	$1,870
Paired Read, 50 Base	$1,925	$2,250
Paired Read, 100 Base	$2,500	$2,950

Minimum DE in a well annotated organism with two conditions (preliminary experiment)

- 2 replicates per condition
- 30+ million reads per sample
- One 1X50 HiSeq lane ($1150)
- Library prep costs ($???)
- Probably around $3000 total

How much sequencing should I do?

Differential expression

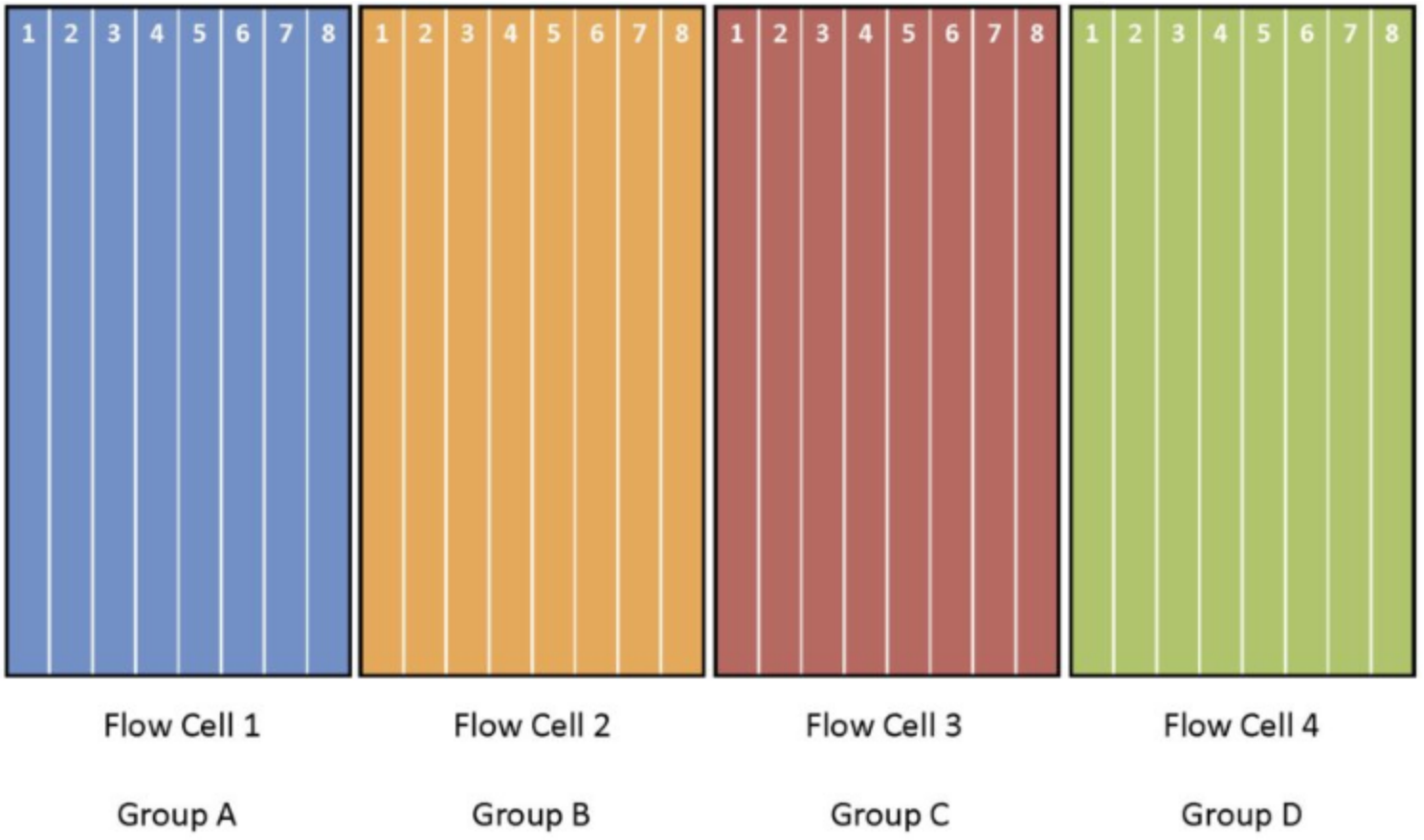
- Replicates more important than depth
- Spend money on biological replicates
- Sacrifice read depth and length
- Human: 20-30 million reads/sample

Pool samples later for novel discovery
Must confirm with other methods

How much sequencing should I do?

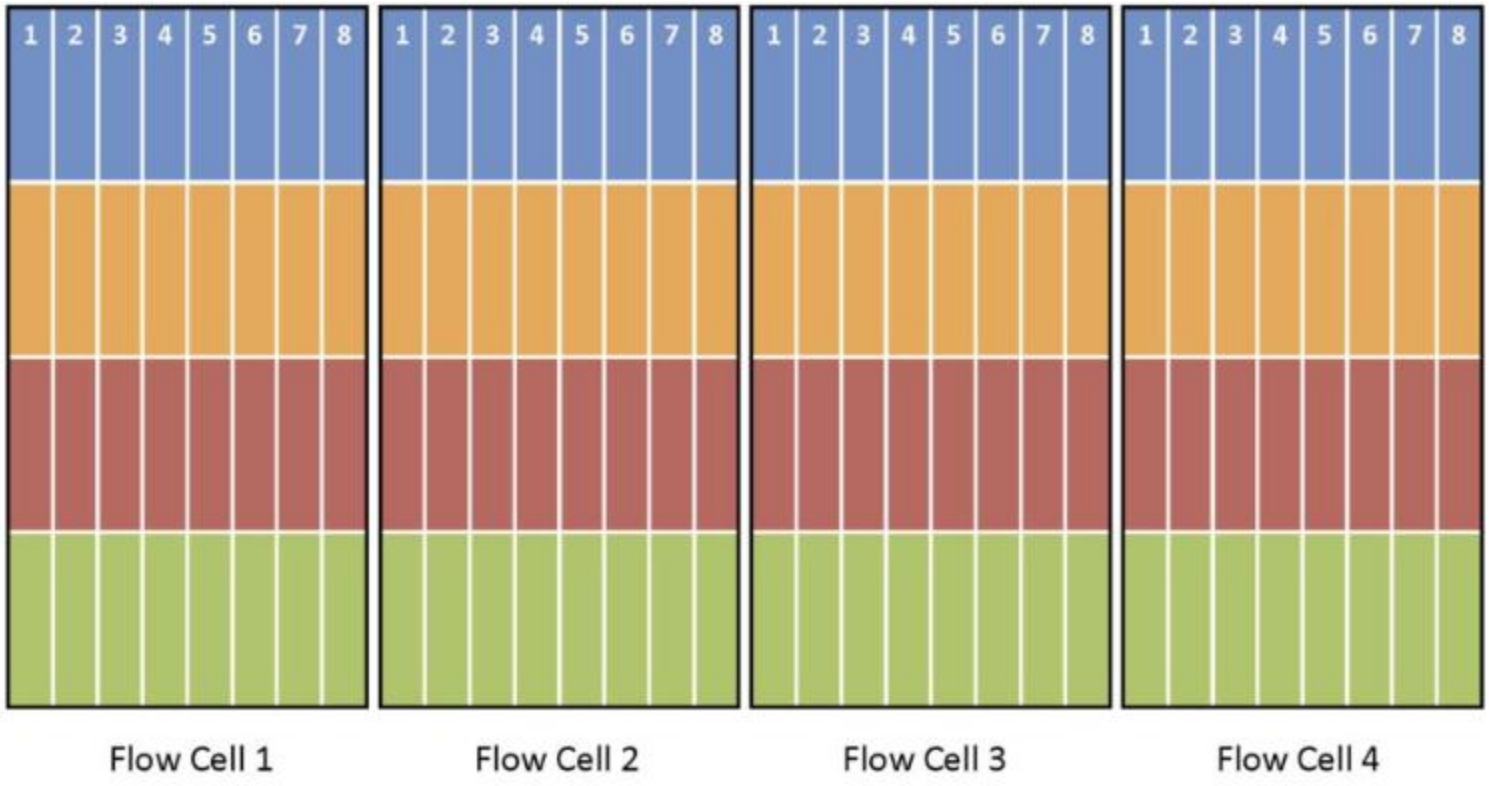
- Discovery
  - Novel genome vs. known genome
  - Depth and read length more important than replicates
  - Spend money on more, longer, paired-end reads
  - Sacrifice replicates (still a good idea!)
  - Human: 200+ million reads/sample

Poor experimental design



Cannot control for lane or flowcell effects

Multiplex for better design



Same sequencing depth

Control for lane and flowcell effects

Scotty – Power Analysis for RNA Seq Experiments

http://euler.bc.edu/marthlab/scotty/scotty.php

An online "tool to assist in the designing of RNA Seq experiments that have adequate power to detect differential expression at the level required to achieve experimental aims."

Review

- Library prep involves: fragmentation, end repair, A-tailing, adapter ligation, size selection, PCR cycle optimization, library quantitation
- Do as much sequencing as you can afford.
- Make sure your sequencing is adequate to answer your question.
- Multiplexing controls for lane and flowcell effects.

Quick break

Lecture Outline

- Acknowledgements
- Computational Biology Initiative
- Course goals/outline
- Cluster test
- Sequencing technologies
- Planning an RNA-seq experiment
- Library prep
- **Design exercises**
- Some helpful resources

Questions to answer

- How will I make the cDNA?
- How will I remove rRNA, gDNA, and mitochondrial sequences?
- How will I prepare the library?
- How much sequencing should I do?

Design an RNA-Seq experiment

What alternative splicing differences are there between human and chimpanzee brains?

Design an RNA-Seq experiment

How does drug treatment affect gene expression in a pathogenic bacteria?

Design an RNA-Seq experiment

How does gene expression differ in 200 FFPE pancreatic cancer samples from human patients?

Design an RNA-Seq experiment

Sequence a ribosome-protected RNA fragment from yeast.

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- Design exercises
- **Some helpful resources**

Indispensable resources

1. Seqanswers

   Online forum for all things NGS

2. BioStar

   Question and answer site about bioinformatics

3. RNA-Seq Blog

   Blog covering industry news, interesting papers, new tools

4. My NCBI

   Get RNA-Seq papers sent to your email

SEQanswers

Online forum for NGS issues

Li, J.-W., et.al. (2012). SEQanswers: An open access community for collaboratively decoding genomes. Bioinformatics.

Create an account if you don't have one!

SEQanswers

Forums

Wiki – Find RNA-Seq software

Instrument Map

Want to keep up with what's new?

Twitter: @agbt Hashtag: #AGBT

RNA-Seq Blog

Other blogs and resources on course website
http://sites.tufts.edu/cbi/resources/rna-seq-course/

Questions? Minute cards!

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